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(71) Applicant (for all designated States except US): AMGEN INC. [US/US]; Amgen Center, One Amgen Center Drive, Thousand Oaks, CA 91320-1789 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BOYLE, William, J. [US/US]; 11678 Chestnut Ridge Street, Moorpark, CA 93021 (US). WOODEN, Scott [US/US]; 1668 Sweet Briar Place, Thousand Oaks, CA 91362 (US).

(74) Agents: ODRE, Steven, M. et al.; Amgen, Inc., Amgen Center, One Amgen Center Drive, Thousand Oaks, CA 91320-1789 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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(54) Title: CHIMERIC OPG POLYPEPTIDES

(57) Abstract

Chimeric polypeptides comprising fusions of an osteoprotegerin dimerization domain to a heterologous sequence are provided. Also provided are nucleic acids encoding the polypeptides, expression vectors and host cells for their production and pharmaceutical compositions comprising the polypeptides.

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CHIMERIC OPG POLYPEPTIDES

Field of the Invention

The invention relates generally to chimeric polypeptides. More particularly, the invention relates to chimeric polypeptides comprising a fusion of an osteoprotegerin dimerization domain to a heterologous sequence. The polypeptides may be used in a variety of diagnostic and therapeutic applications.

Background of the Invention

Cells recognize a variety of signals which 15 modulate growth, differentiation and metabolism. Effectors of cellular functions include small molecular weight organic compounds, carbohydrates, amino acids, peptides and proteins. At present, the best understood signalling process employs secretion of a signalling 20 molecule from one cell to modulate functions of other cells (autocrine regulation). It has also been observed that secreted signalling molecules may also modulate the functions of cells which secrete them (paracrine regulation). The ability of cells to 25 respond to external signals usually requires that the appropriate receptors which bind the signalling molecules be present on the cell surface. Proteinmediated signalling between cells involves binding of growth factors, hormones, cytokines, cell adhesion 30 proteins and the like to cell surface receptors.

As a class of proteins, receptors vary in their structure and mode of signal transduction. They are characterized by having an extracellular domain that is involved in binding a signalling molecule and

cytoplasmic domain which transmits an appropriate intracellular signal. Receptor expression patterns ultimately determine which cells will respond to a given ligand, while the structure of a given receptor dictates the cellular response induced by ligand binding. Receptors have been shown to transmit intracellular signals via their cytoplasmic domains by activating protein tyrosine, or protein serine/threonine phosphorylation (e.g., platelet 10 derived growth factor receptor (PDGFR) or transforming growth factor- β receptor-I (TGF β R-I), by stimulating G-protein activation (e.g., β -adrenergic receptor), and by modulating associations with cytoplasmic signal transducing proteins (e.g., TNFR-1 and Fas/APO) 15 (Heldin, Cell <u>80</u>, 213-223 (1995)).

The tumor necrosis factor receptor (TNFR) superfamily is a group of type I transmembrane proteins which share a conserved cysteine-rich motif which is repeated three to six times in the extracellular domain 20 (Smith, et al. Cell 76, 953-962 (1994)). Collectively, these repeat units form the ligand binding domains of these receptors (Chen et al., Chemistry 270, 2874-2878 The ligands for these receptors are a structurally related group of proteins homologous to 25 $TNF\alpha$. (Goeddel et al. Cold Spring Harbor Symp. Quart. Biol. <u>51</u>, 597-609 (1986); Nagata et al. Science <u>267</u>, 1449-1456 (1995)). TNF α binds to distinct, but closely related receptors, TNFR-1 and TNFR-2. TNFα produces a variety of biological responses in receptor bearing 30 cells, including, proliferation, differentiation, and cytotoxicity and apoptosis (Beutler et al. Ann. Rev. Biochem. <u>57</u>, 505-518 (1988)).

TNFα is believed to mediate acute and chronic inflammatory responses (Beutler et al. <u>ibid</u>). Systemic delivery of TNFα induces septic shock-like syndrome and

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widespread tissue necrosis. Because of this, TNFα may be responsible for the severe morbidity and mortality associated with a variety of infectious diseases, including sepsis. Mutations in FasL, the ligand for the TNFR-related receptor Fas/APO (Suda et al. Cell 75, 1169-1178 (1993)), is associated with autoimmunity (Fisher et al. Cell 81, 935-946 (1995)), while overproduction of FasL may be implicated in druginduced hepatitis. Thus, ligands to the various TNFR-related proteins often mediate the serious effects of many disease states, which suggests that agents that neutralize the activity of these ligands would have therapeutic value.

Soluble TNFR-1 receptors and antibodies that

bind TNFα have been tested for their ability to

neutralize systemic TNFα (Loetscher et al. Cancer Cells

3, 221-226 (1991)). A naturally occuring form of a

secreted TNFR-1 and TNFR-2 mRNA was recently cloned,

and its product tested for its ability to neutralize

TNFα activity in vitro and in vivo (Kohno et al. Proc.

Natl. Acad. Sci. USA 87, 8331-8335 (1990)). The

ability of this protein to neutralize TNFα suggests

that soluble TNF receptors function to bind and clear

TNF thereby blocking the cytotoxic effects on TNFR
bearing cells.

Recombinantly-produced TNF inhibitors have also been taught in the art. For example, EP 393 438 and EP 422 339 teach the amino acid and nucleic acid sequences of a "30kDa TNF inhibitor" (also known as a p55 receptor) and a "40kDa inhibitor" (also known as a p75 receptor) as well as modified forms thereof, e.g., fragments, functional derivatives and variants. EP 393 438 and EP 422 339 also disclose methods for isolating the genes responsible for coding the

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inhibitors, cloning the gene in suitable vectors and cell types, and expressing the gene to produce the inhibitors. Mature recombinant 30kDa TNF inhibitor and mature recombinant 40kDa TNF inhibitor have been demonstrated to be capable of inhibiting TNF (EP 393 438, EP 422 339, PCT Publication No. WO 92/16221 and PCT Publication No. WO 95/34326).

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A recently identified member of the TNFR family, termed Osteoprotegerin (OPG), is a secreted 10 polypeptide which inhibits osteoclast maturation and markedly increases bone density in transgenic mice expressing the OPG polypeptide. OPG inhibited in vitro the formation of mature osteoclasts from hematopoietic progenitor cells and reduced the extent of bone loss in ovariectomized rats (see co-owned and co-pending U.S. 15 Serial Nos. 08/577,788, filed December 22, 1995; 08/706,945, filed September 3, 1996; and 08/771,777 filed December 20, 1996). OPG may have benefit in the treatment of osteopenia. PCT Application No. WO96/26217 discloses a polypeptide termed 20

WO96/26217 discloses a polypeptide termed
Osteoclastogenesis Inhibitory Factor (OCIF) which is
identical to OPG.

OPG comprises two domains having different structural and functional properties. 25 amino-terminal domain spanning residues 22-194 in the mature polypeptide shows homology to other members of the TNFR family, especially TNFR-2, through conservation of cysteine rich domains characteristic of TNFR family members. The carboxy terminal domain 30 spanning residues 194-401 has no significant homology to any known sequences. Unlike a number of other TNFR family members, OPG appears to be exclusively a secreted protein and does not appear to be synthesized as a membrane associated form. Analysis of OPG by 35 reducing and non-reducing gel electrophoresis indicated that the full-length mature polypeptide of 380 amino

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acids formed a dimer having a molecular weight of about 120 kDa as compared to the monomer molecular weight of about 60 kDa. OPG polypeptides having certain truncations in the carboxy terminal domain or substitutions of certain cysteine residues within in 5 the carboxy terminal domain formed dimeric OPG to a lesser extent and had lower biological activity compared to wild-type OPG. However, replacement of part or all of the OPG carboxy terminal domain with an 10 Fc region of IgG restored biological activity in the OPG fusion protein to near normal levels. these observations, the amino-terminal region of OPG appeared to be required for biological activity while the carboxy-terminal domain was important for 15 dimerization. In addition, the biological activity of OPG appeared to be enhanced when the molecule was in dimeric form.

In a therapeutic regimen, it is often desirable to modulate a biological response either by enhancing or blocking a signal received by a receptor. 20 Enhancement of a biological response can involve increasing the affinity of the signalling molecule for a receptor, or increasing the half-life of the molecule in circulation such that it is bound to the receptor for a longer period of time. When the signalling 25 molecule is a polypeptide, enhancement of a biological response may be achieved by constructing analogs which have amino acid sequence changes that increase binding or half-life, derivatives (e.g., polypeptides modified 30 with water soluble polymers) to increase solubility and/or half-life, or chimeric polypeptides (e.g. polypeptides fused to the Fc region of IgG) which increase half-life, solublility and/or modify the aggregation state of the protein in circulation. 35 Similar approaches may be taken to develop therapeutic

proteins which act as antagonists by blocking a

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biological response. In particular, soluble forms of transmembrane receptors which may encompass part or all of the extracellular domains have been used to prevent ligand binding and receptor activation. Soluble receptors have been developed as chemically-modified derivatives and as chimeric polypeptides.

Due to the relatively low inhibition of cytotoxicity exhibited by the 30kDa TNF inhibitor and 40kDa TNF inhibitor (Butler et al. Cytokine 6, 616-623 (1994)), various groups have generated dimers of TNF inhibitor proteins (Butler et al. (1994), supra; and Martin et al. Exp. Neurol. 131, 221-228 (1995)). However, the dimers may generate an antibody response (Martin et al. (1995), supra; and Fisher et al. New Eng. J. Med., 334, 1697-1702 (1996)).

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Generation of chimeric polypeptides has been described in the art. For example, construction of hydrid immunoglobulin molecules by fusion of a ligand binding partner to a human IgG chain is described in U.S. Patent Nos. 5,116,964 and 5, 428,130. Construction of a chimeric polypeptide comprising the extracellular domain of a TNF receptor fused to a mouse IgG heavy chain is described in U.S. Patent No.

5,447,851. Chimeric polypeptides comprising the extracellular domain of a human PDGF receptor fused to dimerizing proteins is described in EP 0 721 983. Multimers of soluble forms of TNF receptors are described in U.S. Patent No. 5,478,925.

While fusion proteins, such as those

30 comprising immunoglobulin constant regions, may have
desirable biological properties, they can elicit an
immune response which limits their usefulness as a
human therapeutic.

Therefore, it is an object of the invention 35 to provide chimeric polypeptides which enhance or block a biological response. Such polypeptides may have

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increased stability, solubility, circulating half-life and decreased immunogenicity.

It is another object of the invention to provide chimeric polypeptides which combine the active region of a signalling molecule with an OPG dimerization domain wherein said chimeric polypeptides will enhance or block a biological response characteristic of the signalling molecule portion of the chimera.

It is another object of the invention to provide OPG chimeric polypeptides which form dimers, trimers and higher multimers which may have advantageous properties such as increased binding affinity, greater stability, and longer circulating half-life compared to monomeric forms.

Summary of the Invention

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The invention provides for chimeric polypeptides comprising fusions of an OPG dimerization domain to a heterologous sequence. Also provided for are nucleic acid sequences encoding the polypeptides, expression vectors and host cells for production of the polypeptides, and pharmaceutical compositions comprising the polypeptides.

25 A heterologous sequence of the invention comprises an amino acid sequence of a cell signalling molecule, such as a receptor, an extracellular domain thereof, and an active fragment, derivative and analog of a receptor or an extraceullular domain. preferred embodiment, heterologous sequences are 30 selected from the family of TNF-like receptors. Such sequences preferentially include functional extracellular ligand binding domains and lack functional transmembrane and cytoplasmic domains. another embodiment, the transmembrane and cytoplasmic 35 domains are deleted in whole or in part.

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understood that heterologous sequences of the invention do not include the amino terminal region of OPG defined by residues 22-194 as shown in U.S. Serial No. 08/577,788 filed December 22, 1995 and hereby incorporated by reference, and do not include related amino acid sequences which, when fused to an OPG dimerization domain, exhibit the biological activity of OPG.

Also encompassed by the invention are

multimeric polypeptides comprising covalently
associated monomers of OPG chimeric polypeptides. The
monomers may have identical heterologous sequences or
different heterologous sequences. In a preferred
embodiment, the multimeric polypeptide is a dimer,
either a heterodimer (different heterologous sequences)
or a homodimer (identical heterologous sequences).

The chimeric polypeptides of the invention are produced by transforming or transfecting host cells with nucleic acids encoding the polypeptide, culturing the host cells, and recovering the polypeptide from the culture. Also provided for are expression vectors and host cells for producing the chimeric polypeptides.

The chimeras are useful for detecting molecules which interact with fused heterologous sequences and thereby identifying potential new receptors and ligands. The compositions of chimeric polypeptides provided herein are useful for treatment of a variety of disorders, for example those related to receptor binding. In one embodiment, compositions comprising TNF/OPG and TNFR/OPG chimeric are used to treat TNF and TNFR mediated disorders, such as inflammation, autoimmune diseases, and disorders related to excessive apoptosis

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Description of the Figures

Figure 1. Amino acid sequences of human, mouse and rat OPG dimerization domains (residues 194-401 of corresponding full-length OPG polypeptides). Conserved cysteine residues implicated in disulfide bond formation are underlined.

Figure 2. Nucleic acid and amino acid sequence of mature, full-length 30 kDa TNF inhibitor.

Figure 3. Nucleic acid and amino acid sequence of mature, full-length 40 kDa TNF inhibitor.

Figure 4. Amino acid sequences of TNFbp/OPG chimeric polypeptides. The TNFbp portion of the chimera is the full-length 30 kDa TNF inhibitor with the leader sequence (underlined) and the additional sequence VKGTEDSGTT at the carboxy terminus. OPG dimerization domains are human OPG residues 194-401, 196-401, 217-401, 248-401 and 304-401. The junction of the TNFbp and OPG sequences creates an Age I restriction site in the DNA sequence and adds a glycine codon (at position 212).

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Figure 5. Gel electrophoresis analysis of TNFbp/OPG chimeric polypeptides. TNFbp/OPG chimeic plasmids were transfected into CHO d-cells. supernatants from serum-free roller bottle harvests were analyzed on a 12% polyacrylamide, Tris-glycine, non-reducing gel. Dimerization patterns were compared to a TNFbp-Fc fusion (lane 1) and TNFbp monomer (lane 8).

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Figure 6. Inhibition of TNF α cytotoxicity on L929 cells. Serum-free conditioned medium samples of TNFbp/Fc and TNFbp/OPG [194-401] fusion polypeptides were serially diluted and assayed for inhibition of TNF α cytotoxicity on L929 cells.

Detailed Description of the Invention

The invention provides for a chimeric polypeptide comprising a fusion of an OPG dimerization domain to a heterologous sequence.

The term "heterologous sequence" refers to an amino acid sequence which is involved in cell signalling and acts to modulate cell growth, differentiation or metabolism. In general,

- heterologous sequences comprise extracellular ligand binding domains of cell surface receptors and their cognate ligands. When present as part of an OPG chimeric polypeptide, a heterologous sequence of the invention comprises about ten or more amino acids in
- length, about 20 or more amino acids in length, about 50 or more amino acids in length, and about 100 or more amino acids in length. A heterologous sequence will be of sufficient size to confer on a chimeric polypeptide a functional property such as receptor binding,
- enzymatic activity, inhibitor activity and the like; however, it is understood that the chimeric polypeptides will not have functional properties identical to OPG although they may share one or more functions in common with OPG. Heterologous sequences
- may encode full-length polypeptides or active fragments, derivatives and analogs thereof.

In preferred embodiments, chimeric OPG polypeptides include heterologous sequences encoding growth factors, cytokines, hormones, cell adhesion molecules and other polypeptide factors which are

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typically secreted. Chimeric OPG polypeptides also include heterologous sequences which encode receptors for growth factors, cytokines, hormones, cell adhesion molecules, and the like, and preferably will include extracellular ligand binding domains from said receptors, and active fragments, derivatives and analogs thereof. The heterologous sequences may or may not be capable of forming dimers or higher aggregates when the sequences are present in a naturally occurring form.

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The "OPG dimerization domain" refers to that portion of the OPG polypeptide which is capable of forming covalently associated multimeric polypeptides. It is understood, however, that chimeric polypeptides 15 comprising an OPG dimerization domain are not restricted to forming dimers, but may form higher multimers as well (trimers, tetramers, etc.) domain may have the amino acid sequence of the human osteoprotegerein dimerization domain, or it may be a 20 fragment, derivative or analog thereof which is capable of forming covalently associated multimers. specifically, an OPG dimerization domain will retain one or more cysteine residues which will allow formation of at least one interchain disulfide bond. 25 In a preferred embodiment, the OPG dimerization domain has the amino acid sequence from about residues 194 to 401 inclusive of human OPG.

As used herein, the term "fragment" comprises a deletion of one or more amino acids in a heterologous sequence or in an OPG dimerization domain. The deletion may occur at the amino terminal end, the carboxy terminal end or in an internal region of the sequence. As used herein, the term "derivative" refers to a modification of the polypeptide backbone of an OPG chimera, either within the OPG dimerization domain or within the heterologous sequence. Said modifications

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include, but are not limited to, attachment of water soluble polymers, hydrophobic moieties, fluorescent tags, enzymatic labels and the like. As used herein, the term "analogs" refers to one or more amino acid substitutions and/or insertions within a polypeptide. Substitutions may involve conservative replacements or non-conservative replacements of amino acids which are known to one skilled in the art. Amino acid insertions may occur at the amino or carboxy terminal ends of either the OPG dimerization domain or the heterolgous sequence or both, or may occur in internal regions.

Polypeptides

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Chimeric polypeptides of the invention comprise a heterologous sequence fused at its carboxy terminus to the amino terminus an OPG dimerization domain or, alternatively, an OPG dimerization domain fused at its carboxy terminus to the amino terminus of a heterologous sequence. Chimeric polypeptides may be constructed as a direct fusion of a heterologous sequence and an OPG dimerization domain or may be constructed with a spacer or adapter region having one or more amino acids inserted between the two portions of the polypeptide. Optionally, the spacer region may encode a protease cleavage site. The precise site of the fusion is not critical and may be varied by one skilled in the art in order to optimize binding charcteristics and/or biological activity of the heterologous sequence.

According to the invention, an OPG dimerization domain may be mammalian in origin (such as from mouse, rat or human) or may be a fragment or analog thereof which is capable of forming covalently associated dimers or higher order multimers. The amino acid sequences of rat, mouse and human OPG dimerization domains span from about residues 194-401 of their

respective full-length OPG polypeptides as shown in Figure 1 (SEQ ID NO:__). Fragments and analogs of an OPG dimerization domain include: deletion or substitution of a cysteine residue at any of positions 5 195, 202, 277, 319 and 400; addition of one or more cysteine residues; rearrangement of the configuration of cysteine residues which may entail a net increase from, a net decrease from, or no change in the number of cysteine residues compared to residues 194-401 of 10 the human OPG dimerization domain; amino-terminal truncations of OPG[194-401], e.g, 195-401, 196-401, and so forth; C-terminal truncations of OPG[194-401], e.g, 194-400, 194-399, and so forth; conservative substitutions of amino acid residues in OPG[194-401] 15 wherein the substitutions comprise replacements with structurally or functionally similar amino acids which are known to one skilled in the art; and any combinations thereof.

Heterologous sequences which form part of a 20 chimeric OPG polypeptide include receptors having known extracellular ligand binding domains. Examples are receptor protein-tyrosine kinases, such as the platelet-derived growth factor receptor (PDGFR) family, fibroblast growth factor receptor (FGFR) family, 25 insulin receptor family, epidermal growth factor receptor (EGFR) family, nerve growth factor (NGFR) family, hepatocyte growth factor family (HGFR), EPH family, AXL family, TIE family, DDR family, ROR family, and other receptor protein tyrosine kinases (see van 30 der Geer et al. Ann. Rev. Cell Biol. 10, 251-337 (1994)). Other examples of receptors having extracellular ligand binding domains include the cytokine receptor superfamily, such as G-CSF, GM-CSF (α and β subunits), MGF, EPO, MGDF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, growth hormone, α -35

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interferon, β -interferon, and γ -interferon receptors, the seven transmembrane domain receptor superfamily, such as acetylcholine, adrenergic, dopamine, thrombin, FSH, gonadotropin, thyrotropoin, clacitonin and parathyroid hormone receptors, and cell adhesion receptors. It is understood that the receptors cited herein are merely examples and that heterologous sequences present in OPG chimeric polypeptides are not limited to the above-mentioned receptors.

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Other heterologous sequences of the invention comprise growth factors, hormones, cytokines, cell adhesion proteins and the like. Also included are corresponding ligands for the receptor protein tyrosine kinases, ligands for cytokine receptors, ligands for seven transmembrane domain receptors, and ligands for cell adhesion receptors.

In a preferred embodiment, the heterologous sequence is a member of the TNF receptor superfamily or is derived from a member of the TNF receptor family. Members include TNFR-1, TNFR-2, TNFrp, NGFR, FasB, 20 CD40, OX40, CD27, CD30, and 4-1BB. Typically the extracellular domains of TNF receptors, or active fragments, derivatives and analogs thereof, are fused to an OPG dimerization domain. Active fragments of TNF receptors will have at least one cysteine rich domain, 25 alternatively two, three or four cysteine rich domains, or alternatively one, two or three cysteine rich domains and a portion thereof, for example, two cysteine rich domains and a portion of a third domain. 30 Activity of a TNF/OPG chimeric polypeptide may include biological activity or ligand binding activity characteristic of a TNF family member which may be evaluated using procedures known to one skilled in the art.

35 Preferred heterologous sequences comprise TNFR-1 or are derived from TNFR-1, and may be

a 30kDa TNF inhibitor, a 40 kDa TNF inhibitor, or a functionally active low molecular weight TNF inhibitor. The nucleic acid and amino acid sequence of mature, full-length 30kDa TNF inhibitor is shown in Figure 2 (SEQ ID NO:__). The nucleic acid and amino acid 5 sequence of mature, full-length 40kDa TNF inhibitor is shown in Figure 3 (SEQ ID NO:__). The low molecular weight TNF inhibitors are modified forms of the 30kDa TNF inhibitor and 40 kDa TNF inhibitor which do not contain the fourth domain (amino acid residues Thr^{127} -10 Thr¹⁶¹ of the 30kDa TNF inhibitor and amino acid residues Pro¹⁴¹-Thr¹⁷⁹ of the 40kDa TNF inhibitor); a portion of the third domain (amino acid residues Asn^{111} -Cys¹²⁶ of the 30kDa TNF inhibitor and amino acid residues Pro123-Lys140 of the 40kDa TNF inhibitor); and, 15 optionally, which do not contain a portion of the first domain (amino acid residues Asp¹-Lys²¹ of the 30kDa TNF inhibitor and amino acid residues Leu1-Lys34 of the 40kDa TNF inhibitor).

The heterologous sequences of the present invention include derivatives of TNFR-1 proteins represented by the formula R_1 -[Cys¹⁹-Cys¹⁰³]- R_2 and R_4 -[Cys³²-Cys¹¹²]- R_5 . These proteins are deletion variants of the 30kDa TNF inhibitor and the 40kDa TNF inhibitor, respectively, and are referred to as "truncated TNFbp(s)".

By " R_1 -[Cys^{19} - Cys^{103}]- R_2 " is meant one or more proteins wherein [Cys^{19} - Cys^{103}] represents residues 19 through 103 of mature, full-length 30kDa TNF inhibitor, the amino acid residue numbering scheme of which is provided in Figure 2 (SEQ ID NO:__) to facilitate the comparison; wherein R_1 represents a methionylated or nonmethionylated amine group of Cys^{19} or of aminoterminus amino acid residue(s) selected from the group:

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С
                IC
               SIC
              NSIC
                     (SEQ ID NO:__)
             NNSIC
                     (SEQ ID NO:_ )
            QNNSIC
                     (SEQ ID NO:__)
           PQNNSIC
                     (SEQ ID NO:__)
          HPQNNSIC
                     (SEQ ID NO:__)
         IHPQNNSIC (SEQ ID NO:__)
        YIHPQNNSIC
                     (SEQ ID NO:__)
       KYIHPQNNSIC
                     (SEQ ID NO:__)
      GKYIHPQNNSIC
                     (SEQ ID NO:__)
     QGKYIHPQNNSIC
                     (SEQ ID NO:___)
    PQGKYIHPQNNSIC
                     (SEQ ID NO:___)
   CPQGKYIHPQNNSIC
                    (SEQ ID NO:__)
  VCPQGKYIHPQNNSIC
                     (SEQ ID NO:___)
 SVCPQGKYIHPQNNSIC
                    (SEQ ID NO:___)
DSVCPQGKYIHPQNNSIC
                     (SEQ ID NO:__);
```

and wherein R_2 represents a carboxy group of Cys^{103} or of carboxy-terminal amino acid residues selected from the group:

```
F
FC
FCC

FCCS (SEQ ID NO:__)
FCCSLC (SEQ ID NO:__)
FCCSLC (SEQ ID NO:__);
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and variants thereof.

Exemplary tumor necrosis factor binding proteins which comprise TNFbp/OPG chimeric polypeptides

of the present invention include the following molecules: NH2-MDSVCPQGKYIHPQNNSIC-[Cys19-Cys103]-FC-COOH (also referred to as 30kDa TNFbp 2.6C105); NH2-MDSVCPQGKYIHPQNNSIC-[Cys19-Cys103]-FNCSL-COOH 5 (also referred to as 30kDa TNFbp 2.6C106); $\mathrm{NH_2}\text{-}\mathrm{MDSVCPQGKYIHPQNNSIC}\text{-}[\mathrm{Cys}^{19}\text{-}\mathrm{Cys}^{103}]\text{-}\mathrm{FNCSL}\text{-}\mathrm{COOH}$ (also referred to as 30kDa TNFbp 2.6N105); NH2- ${\tt MYIHPQNNSIC-[Cys^{19}-Cys^{103}]-FNCSL-COOH}$ (also referred to as 30kDa TNFbp 2.3d8); $NH_2-M-[Cys^{19}-Cys^{103}]-FNCSL-COOH$ 10 (also referred to as 30kDa TNFbp 2.3d18); and $\ensuremath{\text{NH}_2\text{-MSIS-[Cys19-Cys$^{103}]-FNCSL-COOH}}$ (also referred to as 30kDa TNFbp 2.3d15), either methionylated or nonmethionylated, and variants and derivatives thereof. By $R_4-[Cys^{32}-Cys^{112}]-R_5$ is meant one or more proteins wherein [Cys³²-Cys¹¹²] represents residues 15 Cys^{32} through Cys^{112} of mature, full-length $40\mathrm{kDa}$ TNF inhibitor, the amino acid residue numbering scheme of

which is provided in Figure 3 (SEQ ID NO:__) to facilitate the comparison; wherein R₄ represents a

20 methionylated or nonmethionylated amine group of Cys³² or of amino-terminus amino acid residue(s) selected from the group:

C

MC

QMC (SEQ ID NO:___) AQMC TAQMC (SEQ ID NO:__) QTAQMC (SEQ ID NO:___) DQTAQMC (SEQ ID NO:__) YDOTAOMC (SEQ ID NO:___) YYDQTAQMC (SEQ ID NO:__) EYYDQTAQMC (SEQ ID NO:___) REYYDQTAQMC (SEQ ID NO:___) LREYYDQTAQMC (SEQ ID NO:__)

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RLREYYDQTAQMC
                                   (SEQ ID NO:__)
                 CRLREYYDQTAQMC
                                   (SEQ ID NO:___)
               TCRLREYYDQTAQMC
                                   (SEQ ID NO:__)
               STCRLREYYDQTAQMC
                                   (SEQ ID NO:___)
              GSTCRLREYYDQTAQMC
                                   (SEQ ID NO:___)
             PGSTCRLREYYDQTAQMC
                                   (SEQ ID NO: __)
            EPGSTCRLREYYDOTAOMC
                                   (SEQ ID NO: )
           PEPGSTCRLREYYDOTAOMC
                                   (SEQ ID NO:__)
          APEPGSTCRLREYYDQTAQMC
                                   (SEQ ID NO:__)
         YAPEPGSTCRLREYYDOTAOMC
                                   (SEQ ID NO:___)
        PYAPEPGSTCRLREYYDQTAQMC
                                   (SEQ ID NO:___)
       TPYAPEPGSTCRLREYYDQTAQMC
                                   (SEQ ID NO:__)
      FTPYAPEPGSTCRLREYYDOTAOMC
                                   (SEQ ID NO:__)
     AFTPYAPEPGSTCRLREYYDOTAOMC
                                   (SEQ ID NO:___)
    VAFTPYAPEPGSTCRLREYYDQTAQMC
                                   (SEQ ID NO:__)
   QVAFTPYAPEPGSTCRLREYYDQTAQMC
                                   (SEQ ID NO:___)
  AQVAFTPYAPEPGSTCRLREYYDQTAQMC
                                   (SEQ ID NO:__)
 PAQVAFTPYAPEPGSTCRLREYYDQTAQMC
                                   (SEQ ID NO:__)
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMC
                                   (SEQ ID NO:__);
```

and wherein R_5 represents a carboxy group of Cys^{112} or of carboxy-terminal amino acid residues selected from the group:

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RL

RLC

RLCA (SEQ ID NO:__)

RLCAP (SEQ ID NO:__)

RLCAPL (SEQ ID NO:__)

RLCAPLR (SEQ ID NO:__)

RLCAPLRK (SEQ ID NO:__)

RLCAPLRK (SEQ ID NO:__)

(SEQ ID NO:___)

R

RLCAPLRKCR

and variants thereof.

As shown in Example 1, a hybrid DNA molecule encoding TNFbp 4.0, the full-length 30 kDa TNF inhibitor (Figure 2) with the additional sequence VKGTEDSGTT extending from the carboxy terminus, and human OPG [194-401] was constructed. The resulting 5 chimeric polypeptide, termed TNFbp/OPG[194-401] has the amino acid sequence as shown in Figure 4. expression, the mature chimeric polypeptides formed dimers in conditioned medium of transfected host cells as determined by non-reducing SDS-PAGE (see Figure 5). 10 Additional TNFbp fusions were constructed to amino terminal truncations of the human OPG dimerization domain. These constructs are designated TNFbp/OPG[196-401], TNFbp/OPG[217-401], TNFbp/OPG[248-401], and TNFbp/OPG[304-401] and the amino acid sequences are 15 shown in Figure 4. OPG[194-401] has the full complement of five cysteine residues which are involved in covalent association of OPG dimerization domains. OPG[196-401] lacks one cysteine residue at position 20 195, OPG[217-401] and OPG[248-401] lacks a second cysteine residue at position 202, and OPG[304-401] lacks a third cysteine residue at position 277 (see Figure 1 for location of cysteine residues). chimeric polypeptides produced in conditioned medium of 25 transfected CHOd- host cells were analyzed by non-reducing SDS-PAGE (Figure 5). In the L929 cytotoxicity assay, the TNFbp/OPG[194-401] chimera showed activity similar to a TNFbp/Fc chimera (Figure 6).

The invention also provides for chimeric OPG polypeptides which form multimers (i.e., dimers, trimers and higher multimers). Multimers of the invention comprise covalently associated monomeric OPG chimeras wherein the monomers may have identical heterologous sequence or different heterologous sequences. Preferably, the chimeric polypeptides are

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dimers or trimers. Preparations of multimeric polypeptides will be essentially free of monomeric OPG chimeras which are not covalently associated and of inactive multimers. Such preparations are made using techniques available to one skilled in the art

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Modifications of chimeric OPG polypeptides are encompassed by the invention and include post-translational modifications (e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of OPG which may provide 20 additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as 25 polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may 30 include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1kDa and about 100kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules

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will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

10 The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g. EP 0 401 384 herein incorporated by reference 15 (coupling PEG to G-CSF), see also Malik et al. Exp. Hematol. 20, 1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through 20 amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the 25 N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydrl groups may also be used as a reactive group for attaching the polyethylene glycol molecule(s). Preferred for therapeutic purposes is 30 attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire N-terminally chemically modified protein. Using polyethylene
35 glycol as an illustration of the present compositions, one may select from a variety of polyethylene glycol

molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. 5 The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. 10 Selective N-terminal chemically modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available 15 for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

20 The chimeric OPG polypeptides of the invention are isolated and purified from other constituents present in lysates or supernatants of host cells expressing the polypeptides. In one embodiment, the polypeptide is free from association with other 25 human proteins, such as the expression product of a bacterial host cell. Also provided by the invention is a method for the purification of OPG chimeric polypeptides. The purification process may employ one or more standard protein purification steps in an 30 appropriate order to obtain purified protein. chromatography steps can include ion exchange, gel filtration, hydrophobic interaction, reverse phase, chromatofocusing, affinity chromatography employing an anti-OPG antibody or biotin-streptavidin affinity 35 complex and the like. When preparations of selected multimeric OPG chimeras are desired, the purification

method may be carried out to separate species of different aggregation states, for example, separation of monomeric from dimeric OPG chimeras, or separation of dimeric from tetrameric OPG chimeras.

5 Chimeric OPG polypeptides may be used in assays to screen for binding molecules. Examples of such molecules include, but are not limited to, nucleic acids, polypeptides, small molecular weight peptides, carbohydrates, lipids and small molecular weight 10 organic compounds. Assays will employ combining candidate molecules (either purified or unpurified) with chimeric OPG polypeptides under conditions that allowing binding, and measuring the extent of binding to the chimeric polypeptide. Binding measurements are 15 made using detection systems available to one skilled in the art, such as radioactivity, enzymatic activity, fluorescence, and surface plasmon resonance.

Nucleic Acids

20 The invention provides for an isolated nucleic acid encoding a chimeric polypeptide having an OPG dimerization domain fused to a heterologous sequence. The nucleic acids encode a chimeric OPG polypeptide wherein the heterologous sequence is a cell signalling molecule such as a receptor or a receptor 25 ligand. In a preferred embodiment, the heterologous nucleic acid sequence encodes a polypeptide of the TNFR family, or a fragment, derivative or analog thereof, provided however that the heterologous nucleic acid 30 sequence does not encode OPG[22-194] as shown in U.S. Serial No. 08/577,788 filed December 22, 1995, or a homologous sequence which, when fused to an OPG dimerization domain, has the biological activity of OPG.

The nucleic acids of the invention encode chimeric OPG polypeptides selected from the following:

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- a) the nucleic acid sequences which encode the polypeptides shown in Figure 1 (SEQ ID NO: ____) or complementary strands thereof; and
- b) the nucleic acids sequences which hybridize
 under high stringency conditions with the sequences in
 (a), and degenerate sequences thereof,
 provided however that the polypeptides do not have the
 biological activity of OPG. Nucleic acids encoding OPG
 chimeric polypeptides may hybridize over part or all of
 the nucleic acid sequences encoding the OPG
 dimerization domains shown in Figure 1 (SEQ ID NO: _____
).

The conditions for hybridization are generally of high stringency using temperatures,

15 solvents and salt concentrations wherein the hydridizing sequences are about 12-20°C below the melting temperature (T_m) of the perfectly matched duplex. Equivalent stringency to these conditions may be readily ascertained by one skilled in the art by adjusting salt and organic solvent concentrations and temperature. Specific hybridization conditions are described in Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)

Preferred sequences include nucleic acids which encode chimeric OPG polypeptides having rat, mouse and human OPG dimerization domains. DNA encoding human OPG dimerization domain was provided in a full-length human OPG plasmid designated pRcCMV - human OPG and deposited with the American Type Culture Collection, Rockville, MD on December 27, 1995 under accession no. 69969. DNA encoding rat OPG dimerization domain was provided in a full-length rat OPG plasmid designated pMOB-B1.1 and deposited with the American Type Culture Collection, Rockville, MD on December 27,

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1995 under ATCC accession no. 69970. DNA encoding mouse OPG dimerization domain was provided in a full-length mouse OPG plasmid designated pRcCMV-murine OPG and deposited with the American Type Culture

- Collection, Rockville, MD on December 27, 1995 under accession no. 69971. The nucleic acids of the invention will hybridize under stringent conditions to the DNA inserts of ATCC accession nos. 69969, 69970, and 69971.
- In a preferred embodiment, heterologous sequences will comprise nucleic acids encoding TNFR-1, and fragments, derivatives and analogs thereof, such as the TNF 30kDa inhibitor or TNF 40kDa inhibitor.

 Presently preferred heterologous sequences include
 those nucleic acids encoding 30kDa TNFbp 2.6C105, 30kDa TNFbp 2.6C106, 30kDa TNFbp 2.6N105, 30kDa TNFbp 2.3d18, 30kDa TNFbp 2.3d18 and 30kDa TNFbp 2.3d15.

Also provided by the invention are nucleic acids encoding variants of an OPG chimeric polypeptide 20 wherein the variations may be in the heterologous sequence or the OPG dimerization domain or both. The nucleic acid derivatives comprise addition, substitution, insertion or deletion of one or more nucleotides such that the resulting sequences encode 25 chimeric OPG polypeptides comprising one or more amino acid residues which have been added, deleted, inserted or substituted in either the heterologous sequence or the OPG dimerization domain or both. The nucleic acid derivatives may be naturally occurring, such as by 30 splice variation or polymorphism, or may be constructed using site-directed mutagenesis techniques available to the skilled worker. Chimeric OPG polypeptide variants are described in the previous section entitled "Polypeptides" and it is anticipated that nucleic acids encoding all variants disclosed therein, and degenerate 35 molecules thereof, are encompassed by the invention.

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Examples of the nucleic acids of the invention include cDNA, genomic DNA, synthetic DNA and cDNA is obtained from libraries prepared from mRNA isolated from various tissues expressing OPG. humans, tissue sources for OPG include kidney, liver, 5 placenta and heart. Genomic DNA encoding OPG is obtained from genomic libraries which are commercially available from a variety of species. Synthetic DNA is obtained by chemical synthesis of overlapping oligonucleotide fragments followed by assembly of the 10 fragments to reconstitute part or all of the coding region and flanking sequences (see U.S. Patent No. 4,695,623). RNA may be obtained in large quantities use of procaryotic expression vectors which direct high-level synthesis of mRNA, such as vectors using T7 15 promoters and RNA polymerase.

Nucleic acid sequences of the invention are useful for the expression of chimeric OPG polypeptides. Expression may be carried out in transfected host cells for production of recombinant protein in quantities sufficient for diagnostic or therapeutic applications. In addition, chimeric OPG polypeptides may be expressed in vivo and secreted into the circulation to provide therapeutic benefit.

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<u>Vectors</u> and Host Cells

Expression vectors containing nucleic acid sequences encoding OPG fusion proteins, host cells transformed with said vectors and methods for the production of OPG fusion proteins are also provided by the invention. An overview of expression of recombinant proteins is found in Methods of Enzymology v. 185, Goeddel, D.V. ed. Academic Press (1990).

Host cells for the production of OPG fusion proteins include procaryotic host cells, such as <u>E. coli</u>, yeast, plant, insect and mammalian host cells.

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E. coli strains such as HB101 or JM101 are suitable for expression. Preferred mammalian host cells include COS, CHOd-, 293, CV-1, 3T3, baby hamster kidney (BHK) cells and others. Mammalian host cells are preferred when post-translational modifications, such as glycosylation and polypeptide processing, are important for OPG chimera activity. Mammalian expression allows for the production of secreted polypeptides which may be recovered from the growth medium.

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10 Vectors for the expression of OPG chimeric polypeptides contain at a minimum sequences required for vector propogation and for expression of the cloned insert. These sequences include a replication origin, selection marker, promoter, ribosome binding site, enhancer sequences, RNA splice sites and transcription 15 termination site. Vectors suitable for expression in the aforementioned host cells are readily available and the nucleic acids of the invention are inserted into the vectors using standard recombinant DNA techniques. 20 Vectors for tissue-specific expression of OPG chimeric polypeptides are also included. Such vectors include promoters which function specifically in liver, kidney or other organs for production in mice, and viral vectors for the expression of OPG in targeted human 25 cells.

Using an appropriate host-vector system, OPG chimeric polypeptides are produced recombinantly by culturing a host cell transformed with an expression vector containing nucleic acid sequences encoding an OPG chimeric polypeptide under conditions such that the polypeptide is produced, and isolating the product of expression. OPG chimeras are produced in the supernatant of transfected mammalian cells or in inclusion bodies of transformed bacterial host cells. OPG chimeras so produced may be purified by procedures known to one skilled in the art as described below.

Expression vectors for mammalian hosts are exemplified by plasmids such as pDSRa described in PCT Application No. 90/14363; see also Methods in Enzymology vol. 185, D.V. Goeddel, ed. pp. 487-511 for additional examples.

5 A variety of expression vectors are available for bacterial host cells and are described in Methods in Enzymology, ibid. pp. 14-37 and references cited therein. It is anticipated that the specific plasmids and host cells described are for illustrative purposes and that the choice of any specific plasmid and host cell for expression of an OPG chimeric polypeptide will depend upon consideration of a variety of factors by one skilled in the art.

15 Antibodies

Also encompassed by the invention are antibodies specifically binding to an OPG chimeric polypeptide. Antigens for the generation of antibodies may be full-length polypeptides or peptides spanning a 20 portion of the OPG sequence. Immunological procedures for the generation of polyclonal or monoclonal antibodies reactive with OPG are known to one skilled in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual Cold Spring Harbor 25 Laboratory Press, Cold Spring Harbor N.Y. (1988)). Antibodies so produced are characterized for binding specificity and epitope recognition using standard enzyme-linked immunosorbent assays. Antibodies also include chimeric antibodies having variable and 30 constant domain regions derived from different species. In one embodiment, the chimeric antibodies are humanized antibodies having murine variable domains and human constant domains. Also encompassed are complementary determining regions grafted to a human 35 framework (so-called CDR-grafted antibodies). Chimeric and CDR-grafted antibodies are made by recombinant methods known to one skilled in the art. Also encompassed are human antibodies made in mice.

Anti-OPG chimera antibodies of the invention

5 may be used as an affinity reagent to purify OPG from
biological samples. In one method, the antibody is
immobilized on CNBr-activated Sepharose and a column of
antibody-Sepharose conjugate is used to remove OPG from
liquid samples. Antibodies are also used as diagnostic

10 reagents to detect and quantitate OPG in biological
samples by methods described below.

Pharmaceutical compositions

The invention also provides for pharmaceutical compositions comprising a 15 therapeutically effective amount of an OPG chimeric polypeptide together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. The term "therapeutically effective amount" refers to an amount which provides a 20 therapeutic effect for a specified condition and route of administration. The composition may be in a liquid or lyophilized form and comprises a diluent (Tris, acetate or phosphate buffers) having various pH values and ionic strengths, solubilizer such as Tween or 25 Polysorbate, carriers such as human serum albumin or gelatin, preservatives such as thimerosal or benzyl alcohol, and antioxidants such as ascrobic acid or sodium metabisulfite. Also encompassed are 30 compositions comprising OPG chimeric polypeptides modified with water soluble polymers to increase solubility or stability. Compositions may also comprise incorporation of OPG chimeric polypeptides into liposomes, microemulsions, micelles or vesicles 35 for controlled delivery over an extended period of time. Selection of a particular composition will

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depend upon a number of factors, including the condition being treated, the route of administration and the pharmacokinetic parameters desired. A more extensive survey of components suitable for pharmaceutical compositions is found in Remington's Pharmaceutical Sciences, 18th ed. A.R. Gennaro, ed. Mack, Easton, PA (1980).

Compositions of the invention may be administered by injection, either subcutaneous,

intravenous or intramuscular, or by oral, nasal, pulmonary or rectal administration. The route of administration eventually chosen will depend upon a number of factors and may be ascertained by one skilled in the art.

15 Pharmaceutical compositions of chimeric OPG polypeptides are useful for treatment of receptormediated disorders, for example disorders resulting from the function (or lack thereof) of protein tyrosine kinases, cytokine, seven transmembrane domain, and cell 20 adhesion receptors. Disorders resulting from the function (or lack thereof) of the corresponding polypeptide ligands of the above referenced receptors may also be treated. In one embodiment, compositions comprising TNF/OPG chimeras are used to treat TNF-related disorders such as inflammation, autoimmune 25 diseases and conditions marked by excessive apoptosis. Chimeras of the invention may act as agonists to stimulate receptor activation and associated changes in cell activity, or chimeras may be antagonists which 30 block receptor function.

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the nucleic acids of the invention together with a pharmaceutically acceptable adjuvant. Nucleic acid compositions will be

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suitable for delivery to cells and tissues as part of an anti-sense or gene therapy regimen.

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

EXAMPLE 1

Construction and Expression of TNFbp/OPG fusion proteins

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The TNFbp/OPG[196-401] chimeric gene was prepared in a two step PCR process. A first round of PCR was designed to produce overlapping PCR products from each gene. The templates used were plasmids 15 p2302, containing the gene encoding TNFbp 4.0 (Figure 4) fused to the Fc region of human IgG1, and plasmid pRcCMV-human OPG (ATCC accession no. 69969), containing the gene for human OPG. The PCR products were gel purified and used as a template to create the chimeric 20 gene. Primers used for the PCR reactions are as 1275-51 (containing a 5' XbaI site, consensus follows: Kozak and the start of the hTNFbp gene) and 1368-82 (containing a portion of OPG cDNA, an AgeI site and the 3' end of the human TNFbp 4.0 sequence) were used to amplify the TNFbp gene from p2302; 1368-83 (containing 25 the 3' end of TNFbp, an AgeI site and the 5' end of the hOPG C-terminal domain) and 1295-27 (containing a SalI site and the 3' end of the OPG cDNA) were used to amplify the OPG[196-401] gene from pRcCMV-human OPG. A second PCR reaction used primers 1275-51 and 1295-27 to 30 generate the chimeric gene.

The PCR product was cut with XbaI/SalI and subcloned into the pDSR α 2 expression vector to give plasmid p389-1. The expression cassette contains a SV40 early promoter driving the expression of the chimeric gene and also includes an SV40 late intron, an

HTLV translation enhancing signal and an $\alpha 2\text{-FSH}$ polyadenylation signal (DeClerck, et al. J. Biol. Chem. 266, 3893-3899 (1991)). The pDSR $\alpha 2$ vector also contains a DHFR cassette for selection in CHO d- cells.

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Primer Sequences:

1275-51:

(SEQ ID NO:___)

10 5'-CGC TCTAGA CCACC ATG GGC CTC TCC ACC GTG-3'
XbaI Kozak M G L S T V

1368-82:

(SEQ ID NO:___)

15 5'-ACACAGGGTAACATCTAT <u>ACCGGT</u> GGTGCCTGAGTCCTCAG-3' hOPG C-terminus AgeI hTNFbp

1368-83:

(SEQ ID NO:__)

20 5'-CTGAGGACTCAGGCACC <u>ACCGGT</u> ATAGATGTTACCCTGTG-3'

E D S G T T G I D V T L

TNFbp AgeI hOPG C-terminus

1295-27:

25 (SEQ ID NO:___)

5'-CCTCT GTCGAC TA TTA TAA GCA GCTTATTTTCACGGATTG-3'
Sall * * L C.... OPG-->

Other constructs with truncated OPG dimerization doamins were created as follows:

The primer pair for OPG[194-401] was 1295-27 and 1428-89.

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1428-89:

(SEQ ID NO:___)

TCA ACCGGT AAA TGT GGA ATA GAT GTT AC

5 Agel K C G I D V T

The primer pair for OPG[217-401] was 1295-27 and 1388-50.

10 1388-50:

(SEQ ID NO:__)

GTTT \underline{ACCGGT} CCT \underline{AAC} TGG CTT \underline{AGT} GTC \underline{AgeI} \underline{P} \underline{N} \underline{W} \underline{L} \underline{S} \underline{V}

15 The primer pair for OPG[248-401] was 1295-27 and 1388-51.

1388-51:

(SEQ ID NO:__)

20 AGC ACCGGT GAA CAG ACT TTC CAG CTG
Agel E Q T F Q L

The primer pair for OPG[304-401] was 1295-27 and 1388-52.

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1388-52:

(SEQ ID NO:__)

GGAA <u>ACCGGT</u> CCG GGA AAG AAA GTG GG Agel P G K K V G

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The corresponding TNFbp/OPG fusion was constructed by excising the AgeI/SalI OPG fragment from p389-1 and replacing it with AgeI/SalI digested OPG PCR products from the above reactions. The amino acid sequences encoded by the above TNFbp/OPG contructs are shown in Figure 4.

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Transient transfections were performed in COS-7 cells by electroporation. Ten µg of plasmid DNA was electroporated into 2x10⁶ cells in 0.8 mls of DMEM. The electroporations were done in 0.4 cm cuvettes at 1.6 kV, 25 mF and 200 ohms. The electroporated cells were plated in 10-cm dishes in DMEM containing 10% FBS, 1x glutamine/penicillin/streptomycin, 1x non-essential amino acids, 1x Na-pyruvate. The following day the media was changed to media containing only 1% FBS.

After an additional 72 hours, the conditioned media was harvested and 17 μl was electrophoresed on a 12% denaturing, non-reducing gel. These gels were blotted and analyzed by western blots for the presence of monomer and covalently-linked dimers. The primary antibody was anti-TNFbp (R&D systems, AB-225-PB) at a

1:1000 dilution and the secondary antibody was HRP, rabbit anti-goat (Pierce) at a 1:1000 dilution.

Stable transfections were done in CHO dcells by calcium phosphate precipitation (DeClerck et 20 al., supra). The transfection was performed as described except that 20 µg of PvuI linearized plasmid was used with 10 μg of herring sperm carrier DNA and 10 μl of calcium phosphate maximizer (Clontech) to transfect to a 10-cm dish containing approximately 5x10° cells. After 2 weeks in HT- selection, colonies 25 were ring-cloned and expanded into 24-well plates. Once confluent, two day serum-free conditioned media (SFCM) was prepared and analyzed for the expression of TNFbp/OPG fusion protein by western blot. High 30 expressing clones were expanded and grown in roller bottles for 7d SFCM harvests. The results are shown in

Figure 5.

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EXAMPLE 2

Biological Activity of TNFbp/OPG chimeric proteins

WEHI Cytotoxicity Assay

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The WEHI assay is an <u>in vitro</u> cell proliferation assay (Edwards et al. Endocrinology 128,989-996 (1991)). The cell lines are sensitive to TNF- α (i.e., TNF- α is cytotoxic). In the presence of a TNF- α inhibitor, the cells were protected from the cytotoxic effect and thus were able to proliferate.

TNF-sensitive WEHI 164 clone 13 cells are suspended at a concentration of 20×10^4 cells/ml in RPMI (Gibco, Grand Island, NY) medium supplemented with 5% Fetal Calf Serum (Hyclone) and penicillin

- 50U/ml:streptomycin 50 mg/ml. One hundred microliters of this cell suspension are placed in each well of flat-bottomed 96-cell microtiter plates, and the cells are allowed to adhere for 4-6 hours at 37°C in 7% CO₂. Medium is then aspirated, and 0.60 mg/ml actinomycin-D
- 20 (Sigma Chemical Co., St. Louis, MO) is added to each well. A standard curve using serial dilutions at 0, 0.001 0.01, 0.1, 1, 10, 100 U/ml recombinant human TNF is run with each assay. Serially diluted 10-fold concentrations of TNFbp/OPG chimeras from serum-free
- conditioned medium are further diluted in RPMI-1640 medium containing 5% FBS and then added to duplicate wells (50 μ l/well) containing adherent WEHI 164 cells after the addition of recombinant mouse TNF- α . WEHI-164 clone 13 cells are incubated for 18 hours at 37°C
- in 5% CO₂. Maximal killing is determined by adding 0.02% Triton X-100 (TX-100) to test wells. After incubation, 70 μl medium are aspirated, and 50 μl of a 1 mg/mL solution of the organic dye MTT tetrazolium (3-[4,5-dimethylthiozol-2-yl]2,5-diphenyl tetrazolium

35 bromide; Sigma) is added, and cells are incubated for

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an additional 4-6 hours. All supernatants are then removed, and 50 μ l DMF/SDS solution (20% SDS, and 50% N,N dimethylformamide, pH 4.7) is added to each well. The DMF/SDS solution is pipetted up and down several times until all MTT crystals are dissolved, and cells 5 were incubated for an additional 2-22 hours. absorbances (abs) are read on a Vmax reader at 570-650. The percent specific cytotoxicity is calculated from optical densities using the formula: % specific cytotoxicity = 100% X [abs(cells + medium) - abs(cells + sample)]/abs(cells + medium) - abs(cells + TX-100)]. The number of units of TNF in each sample is determined using the percent specific cytotoxicities of the murine standards.

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L929 Cytotoxicity Assay

The L929 cytotoxicity assay is an in vitro cell proliferation assay (Parmely et al. J. Immunol. 151, 389-396 (1993), the disclosure of which is hereby incorporated by reference) which also assesses the 20 cytotoxicity of TNF- α -sensitive killing. The cell lines are sensitive to TNF- α (i.e.; TNF- α is cytotoxic). In the presence of a TNF- α inhibitor, the cells are protected from the cytotoxic effect and thus 25 survive and are able to proliferate.

The L929 cell line was obtained from the American Type Culture Collection (catalog number ATCC CCL 1 NCTC clone 929), as described previously by Parmely et. al. (1993), supra. L929 cells were grown in tissue culture flasks in Dulbecco's MEM with 10 % fetal calf serum (FCS) to 80 % confluence. Cells were trypsinized and seeded at 8,000-10,000 cells/well in 100 ml into Falcon #3072 96 well plates and incubated for 20 to 40 hours at 37 °C in 5% CO,. Samples of TNFbp/Fc or TNFbp/OPG [194-401] polypeptides were

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serially diluted in medium and added in triplicate followed by addition of $TNF\alpha$ to reach a final concentration of 0.5 mg/ml. The cultures were incubated at 37 $^{\circ}\text{C}$ overnight and cell density was measured by crystal violet. Medium was removed by inverting the 96 5 well plates. Cells were fixed in 100 µl 100% methanol for 2 minutes. After removal of methanol the plates were allowed to dry for 10 minutes. 100 µl of 0.10% crystal violet stain in 20% methanol was added and 10 plates were Ststained for 10 minutes at room temperature. Excess stain was removed by inverting plates. Plates were washed by dipping three times in ice-cold distilled water and excess water was removed from the wells by gently blotting plates on a tissue. 15 100 μl of 100% methanol was added to stained cells and optical density was measured at 595 nm. Media control reactions contained L929 cells and medium alone, and TNF control reactions contained L929 cells with 0.5 $ng/ml TNF\alpha$.

The activity in this assay of TNFbp/OPG fusions constructed as described in Example 1 is shown in Figure 6.

* *

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While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

SEQUENCE LISTING

5	(1) GENE	RAL INFORMATION:
J	(i)	APPLICANT: Amgen Inc.
10	(ii)	TITLE OF INVENTION: CHIMERIC OPG POLYPEPTIDES
	(iii)	NUMBER OF SEQUENCES: 87
15 20	(iv)	CORRESPONDENCE ADDRESS: (A) ADDRESSEE: Amgen Inc. (B) STREET: 1840 Dehavilland Drive (C) CITY: Thousand Oaks (D) STATE: California (E) COUNTRY: USA (F) ZIP: 91320-1789
25	(v)	COMPUTER READABLE FORM: (A) MEDIUM TYPE: Floppy disk (B) COMPUTER: IBM PC compatible (C) OPERATING SYSTEM: PC-DOS/MS-DOS (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
23	(vi)	CURRENT APPLICATION DATA:
30	(V17	(A) APPLICATION DATA: (B) FILING DATE: (C) CLASSIFICATION:
35	(viii)	ATTORNEY/AGENT INFORMATION: (A) NAME: Winter, Robert B. (C) REFERENCE/DOCKET NUMBER: A-452
	(2) INFO	RMATION FOR SEQ ID NO:1:
40	(i)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 4 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
45	(ii)	MOLECULE TYPE: protein
50	(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:1:
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55	(2) INFO	RMATION FOR SEQ ID NO:2:
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	(ii)	MOLECULE TYPE: protein

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		Pro Gln Asn Asn Ser Ile Cys 1 5
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		Ile His Pro Gln Asn Asn Ser Ile Cys 1 5
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10		(ii) 1	MOLECUI	E TYE	PE: p	rote	in									
15		(xi) s	SEQUENC	E DES	CRIP	TION	: SE	EQ II	ONO:	:13:						
		Val (Cys Pro	Gln	Gly 5	Lys '	Tyr	Ile	His	Pro 10	Gln	Asn	Asn	Ser	Ile 15	Cys
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30		(ii) I	MOLECUI	LE TYI	?E: p	rote	in									
		(xi)	SEQUENC	E DES	SCRIP	TION	: SE	EQ II	ои о	:14:						
35		Ser \	Val Cys	s Pro	Gln 5	Gly	Lys	Tyr	Ile	His 10	Pro	Gln	Asn	Asn	Ser 15	Ile
		Cys														
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50		(ii) I	MOLECUI	LE TYI	PE: p	rote	in									
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		Phe Cys Cys Ser Leu 1 5
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		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
50		Phe Cys Cys Ser Leu Cys 1 5
	(2)	INFORMATION FOR SEQ ID NO:19:
55		(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 7 amino acids(B) TYPE: amino acid(C) STRANDEDNESS: single
60		(D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein

		(X1) SEQUENCE DESCRIPTION: SEQ ID NO:19:
5		Phe Cys Cys Ser Leu Cys Leu 1
	(2)	INFORMATION FOR SEQ ID NO:20:
10		 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 4 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
15		(ii) MOLECULE TYPE: protein
2.0		
20		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
25		Ala Gln Met Cys 1
2 3	(2)	INFORMATION FOR SEQ ID NO:21:
30		(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 5 amino acids(B) TYPE: amino acid(C) STRANDEDNESS: single(D) TOPOLOGY: linear
35		(ii) MOLECULE TYPE: protein
40		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
		Thr Ala Gln Met Cys 1 5
45	(2)	INFORMATION FOR SEQ ID NO:22:
#3		(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 6 amino acids(B) TYPE: amino acid
50		<pre>(C) STRANDEDNESS: single (D) TOPOLOGY: linear</pre>
		(ii) MOLECULE TYPE: protein
55		
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
60		Gln Thr Ala Gln Met Cys 1 5
	(2)	INFORMATION FOR SEO ID NO:23:

5		(1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 7 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
		(ii) MOLECULE TYPE: protein
10		
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
15		Asp Gln Thr Ala Gln Met Cys 1 5
	(2)	INFORMATION FOR SEQ ID NO:24:
20		(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 8 amino acids(B) TYPE: amino acid(C) STRANDEDNESS: single(D) TOPOLOGY: linear
25		(ii) MOLECULE TYPE: protein
30		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
-		Tyr Asp Gln Thr Ala Gln Met Cys 1 5
35	(2)	INFORMATION FOR SEQ ID NO:25:
40		(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 9 amino acids(B) TYPE: amino acid(C) STRANDEDNESS: single(D) TOPOLOGY: linear
		(ii) MOLECULE TYPE: protein
45		
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
50		Tyr Tyr Asp Gln Thr Ala Gln Met Cys 1 5
	(2)	INFORMATION FOR SEQ ID NO:26:
55		(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 10 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single
60		(D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein

		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
5		Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys 1 5 10
	(2)	INFORMATION FOR SEQ ID NO:27:
10		 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 11 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
15		(ii) MOLECULE TYPE: protein
20		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
25		Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys 1 5 10
	(2)	INFORMATION FOR SEQ ID NO:28:
30		 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 12 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
35		(ii) MOLECULE TYPE: protein
40		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28: Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys 1 5 10
	(2)	INFORMATION FOR SEQ ID NO:29:
45		(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 13 amino acids
50		(B) TYPE: amino acid(C) STRANDEDNESS: single(D) TOPOLOGY: linear
		(ii) MOLECULE TYPE: protein
55		
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
60		Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys 1 10
	(2)	INFORMATION FOR SEO ID NO.30.

5		(1)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 14 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
		(ii)	MOLECULE TYPE: protein
10			
		(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:30:
15		Cys 1	Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys 5 10
	(2)	INFO	RMATION FOR SEQ ID NO:31:
20		(i)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 15 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
25		(ii)	MOLECULE TYPE: protein
30		(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:31:
		Thr 1	Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys 5 10 15
35	(2)	INFO	RMATION FOR SEQ ID NO:32:
40		(i)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 16 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
		(ii)	MOLECULE TYPE: protein
45			
		(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:32:
50		Ser 1	Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys 5 10 15
55	(2)	INFO	RMATION FOR SEQ ID NO:33:
33		(i)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single
60			(D) TOPOLOGY: linear
		(ii)	MOLECULE TYPE: protein

5		(xi)	SEQU	JENCE	DE	SCŖI	OITS	1: SI	EQ II	ои о	:33:						
J		Gly 1	Ser	Thr	Cys	Arg 5	Leu	Arg	Glu	Tyr	Tyr 10	Asp	Gln	Thr	Ala	Gln 15	Met
10		Суз															
	(2)	INFO	RMATI	ON F	OR :	SEQ :	ID NO	0:34	:								
15		(i)	(A) (B) (C)	JENCE LEN TYF STR	IGTH PE: 8 RAND	: 18 amin EDNE	amin o ac: SS: s	no ad id sing:	cids								
20		(ii)	MOLI	ECULE	TY:	PE:]	prote	ein									
25		(xi)	SEQ	JENCE	E DE	SCRI	PTIO	N: S	EQ I	D NO	:34:						
		Pro 1	Gly	Ser	Thr	Cys 5	Arg	Leu	Arg	Glu	Tyr 10	Tyr	Asp	Gln	Thr	Ala 15	Gln
3 0		Met	Суѕ														
	(2)	INFO	RMAT:	ION F	FOR :	SEQ	ID N	0:35	:								
35		(i)	(A) (B) (C)	JENCE) LEN) TYE) STF	IGTH PE: RAND	: 19 amin EDNE	amin o ac: SS: :	no a id sing	cids								
40		(ii)		ECULE													
_																	
45		(xi)	SEQ	JENCE	E DE	SCRI	PTIO	N: S	EQ I	ои о	:35:						
50		Glu 1	Pro	Gly	Ser	Thr 5	Cys	Arg	Leu	Arg	Glu 10	Tyr	Tyr	Asp	Gln	Thr 15	Ala
50		Gln	Met	Cys										•			
55	(2)	INFO	RMAT:	ION E	FOR	SEQ	ID N	0:36	:								
ر ر		(i)	(A)	JENCE) LEN) TYE	IGTH	: 20	ami	no a	S: cids								
60			(C) STF) TOF	RAND	EDNE	SS:	sing	le								
		(ii)	MOL	ECULE	YT 3	PE:	prot	ein		•							

5		(xi)	SEQU	ENCE	DES	SCRII	OITS	N: S1	EQ II	D NO	:36:						
J		Pro 1	Glu	Pro	Gly	Ser 5	Thr	Cys	Arg	Leu	Arg 10	Glu	Tyr	Tyr	Asp	Gln 15	Thr
10		Ala	Gln	Met	Cys 20												
	(2)	INFO	RMATI	ON E	FOR S	SEQ :	ID N	0:37	:								
15		(i)	(B) (C)	LEN TYI STI	IGTH PE: 8 RANDI	: 21 amino EDNE:	TERI: amin ac: SS: line	no ao id sing:	cids								
20		(ii)	MOLE	CULE	E TY	PE:]	prote	ein									
25		(xi)	SEQU	JENCI	E DE	SCRI	PTIO	N: S	EQ I	D NO	:37:						
		Ala 1	Pro	Glu	Pro	Gly 5	Ser	Thr	Cys	Arg	Leu 10	Arg	Glu	Tyr	Tyr	Asp 15	Gln
30		Thr	Ala	Gln	Met 20	Cys											
	(2)	INFO	RMATI	ON I	FOR :	SEQ :	ID N	0:38	:								
35		(i)	(B)	LEI TYI STI	NGTH PE: R RAND	: 22 amin EDNE	TERI ami: o ac SS: line	no a id sing	cids								
40		(ii)	MOLE	ECULI	E TY	PE:]	prot	ein									
45																	
			SEQU														
50		Tyr 1	Ala	Pro	Glu	Pro 5	Gly	Ser	Thr	Cys	Arg 10	Leu	Arg	Glu	Tyr	Tyr 15	Asp
30		Gln	Thr	Ala	Gln 20	Met	Cys										-
55	(2)	INFO	RMATI	ON I	FOR	SEQ	ID N	0:39	:								
- -		(i)	(B)	LEI	NGTH PE:	: 23 amin	ami o ac	no a id	cids						•		
60							SS: line		le								
		(ii)	MOLE	ECULI	Е ТҮ	PE:	prot	ein									

5		(xi)	SEQU	JENCE	E DES	SCRII	PTIO	N: SI	EQ II	ои с	:39:						
J		Pro 1	Tyr	Ala	Pro	Glu 5	Pro	Gly	Ser	Thr	Cys 10	Arg	Leu	Arg	Glu	Tyr 15	Tyr
10		Asp	Gln	Thr	Ala 20	Gln	Met	Суѕ									
	(2)	INFO	RMATI	ON E	FOR S	SEQ :	ID N	0:40	:								
15		(i)	(B) (C)	JENCE LEN TYE STE TOE	IGTH: PE: 8 RANDI	: 24 amino EDNES	amin o ac: SS: s	no ad id sing	cids								
20		(ii)	MOLE	CULE	TYI	PE: 1	prote	ein									
25		(xi)	SEQU	JENCE	E DES	SCRI	PTIO	N: SI	EQ II	ONO:	:40:						
		Thr 1	Pro	Tyr	Ala	Pro 5	Glu	Pro	Gly	Ser	Thr 10	Cys	Arg	Leu	Arg	Glu 15	Tyr
3 0		Tyr	Asp	Gln	Thr 20	Ala	Gln	Met	Cys								
	(2)	INFO	RMATI	ON E	FOR S	SEQ :	ID N	0:41	:								
35		(i)	(B) (C)	JENCE LEN TYI STE	NGTH: PE: 8 RANDI	: 25 amino EDNES	amin o ac: SS: :	no ad id sing!	cids								
40		(ii)	MOLE	ECULI	TYI	PE: 1	prote	ein									
45		(xi)	SEQU	JENCI	E DES	CRI	PTIO	N: S1	EQ II	ои о	:41:						
50		Phe 1	Thr	Pro	Tyr	Ala 5	Pro	Glu	Pro	Gly	Ser 10	Thr	Cys	Arg	Leu	Arg 15	Glu
		Tyr	Tyr	Asp	Gln 20	Thr	Ala	Gln	Met	Cys 25							
55	(2)	INFO	RMAT	CON E	FOR S	SEQ :	ID N	0:42	:								
<i></i>		(i)	(B)	LEI TYI	NGTH PE: 8	: 26	amino aci	no ao id	cids								٠.
60			(C)	STI TOI	RANDI	EDNE	ss:	sing:	le								
		(ii)	MOLI	ECULI	E TY	PE:]	prot	ein									

5		(xi)	SEQU	JENCE	E DES	CŖI	OITS	1: SI	EQ II	ON C	:42:						
J		Ala 1	Phe	Thr	Pro	Tyr 5	Ala	Pro	Glu	Pro	Gly 10	Ser	Thr	Cys	Arg	Leu 15	Arg
10		Glu	Tyr	Tyr	Asp 20	Gln	Thr	Ala	Gln	Met 25	Cys						
	(2)	INFO	RMATI	ON E	FOR S	SEQ 1	D NO	0:43	:								
15		(i)	(B)	LEN TYI STI	E CHA NGTH: PE: & RANDI POLOG	: 27 amino EDNES	amin aci	no ad id sing:	cids							·	
20		(ii)	MOLE	ECULI	E TYI	PE: 1	prote	ein									
25		(xi)	SEQU	JENCI	E DES	SCRI	PTIO	N: S	EQ II	ои с	:43:						
		Val 1	Ala	Phe	Thr	Pro 5	Tyr	Ala	Pro	Glu	Pro 10	Gly	Ser	Thr	Суз	Arg 15	Leu
30		Arg	Glu	Tyr	Tyr 20	Asp	Gln	Thr	Ala	Gln 25	Met	Cys	/				
	(2)	INFO	RMAT:	ION I	FOR :	SEQ :	ID N	0:44	:								
35 40		(i)	(B)	LEI TYI STI	E CHANGTH PE: 6 RANDI POLO	: 28 amine EDNE:	amin o ac: SS: :	no a id sing	cids								
40		(ii)	MOLI	ECULI	E TY	PE:]	prot	ein									•
45																	
4		(xi)	SEQ	JENC:	E DE	SCRI	PTIO	N: S	EQ I	ои о	:44:						
50		Gln 1	Val	Ala	Phe	Thr 5	Pro	Tyr	Ala	Pro	Glu 10	Pro	Gly	Ser	Thr	Cys 15	Arg
		Leu	Arg	Glu	Tyr 20	Tyr	Asp	Gln	Thr	Ala 25	Gln	Met	Cys				
55	(2)	INFO	RMAT:	ION 1	FOR a	SEQ :	ID N	0:45	:								
~ ~		(i)	(B)) LEI) TY:	NGTH PE:	: 29 amin	ami: o ac	no a id	cids								
60			(C)) ST	RAND: POLO	EDNE GY:	SS: line	sing ar	le								
		(ii)	MOL	ECUL:	E TY	PE:	prot	ein									

5		(xi)	SEQU	JENCI	E DES	SCŖII	OITS	1: SI	EQ II	ОИ С	:45:						
J		Ala 1	Gln	Val	Ala	Phe 5	Thr	Pro	Tyr	Ala	Pro 10	Glu	Pro	Gly	Ser	Thr 15	Cys
10		Arg	Leu	Arg	Glu 20	Tyr	Tyr	Asp	Gln	Thr 25	Ala	Gln	Met	Cys			
	(2)	INFO	RMATI	ON I	FOR S	SEQ :	ID NO	0:46	:								
15		(i)	(B) (C)	LEI TYI STI	E CHA NGTH: PE: 8 RANDI POLO	: 30 amino EDNES	amir o aci	no ao id sing:	cids								
20		(ii)	MOLE	ECULI	Ξ ТҮ1	PE: 1	prote	ein									
25		(xi)	SEOI	IENCI	e Dec	SCBT1	יירים	cı	ר דו) NO	. 16.						
												Dwo	G1	Deed	01		m1
		1	ALG	O111	var	5	File	IIII	PIO	тАт	10	PIO	GIU	Pro	GIY	Ser 15	Thr
30		Cys	Arg	Leu	Arg 20	Gļu	Туr	Tyr	Asp	Gln 25	Thr	Ala	Gln	Met	Cys 30		
	(2)	INFO	RMATI	ON 1	FOR :	SEQ :	ID N	0:47	:								
35		(i)	SEQU (A) (B) (C) (D)	LEI TYI	E CHANGTH PE: 6 RANDI POLO	: 31 amin EDNE:	amino ac: SS: 8	no ao id sing:	cids								
40		(ii)	MOLI	ECULI	E TY	PE:]	prote	ein									
4 5																	
45		(xi)	SEQU	JENC	E DE	SCRI	PTIO	N: S	EQ I	ои о	:47:						
50		Leu 1	Pro	Ala	Gln	Val 5	Ala	Phe	Thr	Pro	Tyr 10	Ala	Pro	Glu	Pro	Gly 15	Ser
		Thr	Cys	Arg	Leu 20	Arg	Glu	Tyr	Tyr	Asp 25	Gln	Thr	Ala	Gln	Met 30	Сув	
55	(2)	INFO	RMAT:	ON	FOR :	SEQ :	ID N	0:48	:								
		(i)	(B)	LEI	NGTH PE: a	: 4 amin	amino	o ac: id	ids								
60			(D)	TO	RAND: POLO	EUNE:	line	sıng. ar	те								
		(ii)	MOL	ECUL:	E TY	PE:]	prot	ein									

5		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:
J		Arg Leu Cys Ala 1
L O	(2)	INFORMATION FOR SEQ ID NO:49:
15		 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
		(ii) MOLECULE TYPE: protein
		(11) Modelond IIII. procein
20		
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:
25		Arg Leu Cys Ala Pro 1 5
	(2)	INFORMATION FOR SEQ ID NO:50:
30	•	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 6 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
35		(ii) MOLECULE TYPE: protein
40		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:
		Arg Leu Cys Ala Pro Leu 1 5
45	(2)	INFORMATION FOR SEQ ID NO:51:
50		(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 7 amino acids(B) TYPE: amino acid(C) STRANDEDNESS: single
		(D) TOPOLOGY: linear
55		(ii) MOLECULE TYPE: protein
,,		
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:
60		Arg Leu Cys Ala Pro Leu Arg 1 5
	(2)	INFORMATION FOR SEQ ID NO:52:

5		(1)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 8 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
10		(ii)	MOLECULE TYPE: protein
			SEQUENCE DESCRIPTION: SEQ ID NO:52:
15		Arg 1	Leu Cys Ala Pro Leu Arg Lys 5
	(2)	INFOR	RMATION FOR SEQ ID NO:53:
20		(i)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 9 amino acids (B) TYPE: amino acid
25			<pre>(C) STRANDEDNESS: single (D) TOPOLOGY: linear</pre>
23		(ii)	MOLECULE TYPE: protein
30			
		(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:53:
35		Arg 1	Leu Cys Ala Pro Leu Arg Lys Cys 5
J J	(2)	INFOR	RMATION FOR SEQ ID NO:54:
40		(i)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 10 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
45		(ii)	MOLECULE TYPE: protein
50		(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:54:
50		Arg 1	Leu Cys Ala Pro Leu Arg Lys Cys Arg 5 10
55	(2)	INFOR	RMATION FOR SEQ ID NO:55:
-		(i)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 32 base pairs
60			(B) TYPE: nucleic acid(C) STRANDEDNESS: single(D) TOPOLOGY: linear
		(ii)	MOLECULE TYPE: CDNA

(B) LOCATION: 1532	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:	
CGCTCTAGAC CACC ATG GGC CTC TCC ACC GTG Met Gly Leu Ser Thr Val 1 5	32
(2) INFORMATION FOR SEQ ID NO:56:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 6 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: protein	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:	
25 Met Gly Leu Ser Thr Val 1 5	
(2) INFORMATION FOR SEQ ID NO:57:	
30 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 41 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: cDNA	
40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:	
ACACAGGGTA ACATCTATAC CGGTGGTGCC TGAGTCCTCA G	41
45 (2) INFORMATION FOR SEQ ID NO:58:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 40 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: cDNA	
(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 340	
60	

	(X1) SEQUENCE DESCRIPTION: SEQ ID NO:58:	
5	CT GAG GAC TCA GGC ACC GGT ATA GAT GTT ACC CTG TG Glu Asp Ser Gly Thr Thr Gly Ile Asp Val Thr Leu 1 5 10	40
	(2) INFORMATION FOR SEQ ID NO:59:	
10	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 12 amino acids(B) TYPE: amino acid(D) TOPOLOGY: linear	
15	(ii) MOLECULE TYPE: protein	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:	
20	Glu Asp Ser Gly Thr Thr Gly Ile Asp Val Thr Leu 1 5 10	
	(2) INFORMATION FOR SEQ ID NO:60:	
25	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 40 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear 	
30	(ii) MOLECULE TYPE: cDNA	
35	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:	
	CCTCTGTCGA CTATTATAAG CAGCTTATTT TCACGGATTG	40
40	(2) INFORMATION FOR SEQ ID NO:61: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 29 base pairs (B) TYPE: nucleic acid	
45	(C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
50	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1029	
55	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:	
60	TCAACCGGT AAA TGT GGA ATA GAT GTT AC Lys Cys Gly Ile Asp Val 1 5	29

	(2) INFORMATION FOR SEQ ID NO:62:	
5	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 6 amino acids(B) TYPE: amino acid(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: protein	
10	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:	
	Lys Cys Gly Ile Asp Val 1 5	
15	(2) INFORMATION FOR SEQ ID NO:63:	
20	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 28 base pairs(B) TYPE: nucleic acid(C) STRANDEDNESS: single(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
25	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1128	
30	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:	
35	GTTTACCGGT CCT AAC TGG CTT AGT GTC Pro Asn Trp Leu Ser Val 1 5	28
	(2) INFORMATION FOR SEQ ID NO:64:	•
40	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 6 amino acids(B) TYPE: amino acid(D) TOPOLOGY: linear	
45	(ii) MOLECULE TYPE: protein	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:	
50	Pro Asn Trp Leu Ser Val 1 5	
	(2) INFORMATION FOR SEQ ID NO:65:	
55	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 27 base pairs(B) TYPE: nucleic acid(C) STRANDEDNESS: single(D) TOPOLOGY: linear	
60	(ii) MOLECULE TYPE: cDNA	

	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1027	
5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:	
	AGCACCGGT GAA CAG ACT TTC CAG CTG	27
10	Glu Gln Thr Phe Gln Leu 1 5	
	(2) INFORMATION FOR SEQ ID NO:66:	
15	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 6 amino acids(B) TYPE: amino acid(D) TOPOLOGY: linear	
20	(ii) MOLECULE TYPE: protein	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:	
25	Glu Gln Thr Phe Gln Leu 1 5	
	(2) INFORMATION FOR SEQ ID NO:67:	
30	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 27 base pairs(B) TYPE: nucleic acid(C) STRANDEDNESS: single(D) TOPOLOGY: linear	
35	(ii) MOLECULE TYPE: cDNA	
40	(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 1127	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:	
45	GGAAACCGGT CCG GGA AAG AAA GTG GG Pro Gly Lys Lys Val 1 5	27
50	(2) INFORMATION FOR SEQ ID NO:68:	
55	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 5 amino acids(B) TYPE: amino acid(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: protein	
60	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:	
	Pro Gly Lys Lys Val	

	(2)	INFO	RMATI	ON E	FOR S	SEQ]	DN C):69:	:								
5		(i)	(B) (C)	LEN TYP STP	NGTH: PE: & RANDE	ARACT : 208 amino EDNES GY:]	3 ami 3 aci 3S: s	ino a id singl	cids	3							
10		(ii)	MOLE	ECULI	E TYI	?E: p	prote	ein									
15		(xi)	SEQU	JENCI	E DES	SCRII	OITS	1: SI	EQ II	ои с	:69:						
13		Asn 1	Cys	Gly	Ile	Asp 5	Val	Thr	Leu	Cys	Glu 10	Glu	Ala	Phe	Phe	Arg 15	Phe
20		Ala	Val	Pro	Thr 20	Lys	Ile	Ile	Pro	Asn 25	Trp	Leu	Ser	Val	Leu 30	Val	Asr
		Ser	Leu	Pro 35	Gly	Thr	Lys	Val	Asn 40	Ala	Glu	Ser	Val	Glu 45	Arg	Ile	Lys
25		Arg	Arg 50	His	Ser	Ser	Gln	Glu 55	Gln	Thr	Phe	Gln	Leu 60	Leu	Lys	Leu	Tr
30		Lys 65	His	Gln	Asn	Arg	Asp 70	Gln	Glu	Met	Val	Lys 75	Lys	Ile	Ile	Gln	Asr 80
		Ile	Asp	Leu	Cys	Glu 85	Ser	Ser	Val	Gln	Arg 90	His	Ile	Gly	His	Ala 95	Asr
35		Leu	Thr	Thr	Glu 100	Gln	Leu	Arg	Ile	Leu 105	Met	Glu	Ser	Leu	Pro 110	Gly	Lys
		Lys	Ile	Ser 115	Pro	Asp	Glu	Ile	Glu 120	Arg	Thr	Arg	Lys	Thr 125	Cys	Lys	Pro
40		Ser	Glu 130	Gln	Leu	Leu	Lys	Leu 135	Leu	Ser	Leu	Trp	Arg 140	Ile	Lys	Asn	Gly
45		Asp 145	Gln	Asp	Thr	Leu	Lys 150	Gly	Leu	Met	Tyr	Ala 155	Leu	Lys	His	Leu	Lys 160
		Ala	Tyr	His	Phe	Pro 165	Lys	Thr	Val	Thr	His 170	Ser	Leu	Arg	Lys	Thr 175	Ile
50		Arg	Phe	Leu	His 180	Ser	Phe	Thr	Met	Tyr 185	Arg	Leu	Tyr	Gln	Lys 190	Leu	Phe
		Leu	Glu	Met 195	Ile	Gly	Asn	Gln	Val 200	Gln	Ser	Val	Lys	Ile 205	Ser	Суѕ	Let
55	(2)	INFO	RMAT:	ION 1	FOR :	SEQ :	ID N	0:70	:								
60		(i)	(B)) LEI) TYI) STI	NGTH PE: a RAND	ARACT 200 amin EDNE	8 am: o ac: SS: :	ino a id sing:	acid	s							

(ii) MOLECULE TYPE: protein

(ii) MOLECULE TYPE: protein

5																
J	(xi)	SEQU	JENCI	E DES	SCRIE	OITS	1: SI	EQ II	ON O	:70:						
10	Lys 1	Cys	Gly	Ile	Asp 5	Val	Thr	Leu	Cys	Glu 10	Glu	Ala	Phe	Phe	Arg 15	Phe
	Ala	Val	Pro	Thr 20	Lys	Ile	Ile	Pro	Asn 25	Trp	Leu	Ser	Val	Leu 30	Val	Asp
15	Ser	Leu	Pro 35	Gly	Thr	Lys	Val	Asn 40	Ala	Glu	Ser	Val	Glu 45	Arg	Ile	Lys
	Arg	Arg 50	His	Ser	Ser	Gln	Glu 55	Gln	Thr	Phe	Gln	Leu 60	Leu	Lys	Leu	Trp
20	Lys 65	His	Gln	Asn	Arg	Asp 70	Gln	Glu	Met	Val	Lys 75	Lys	Ile	Ile	Gln	Asp 80
25	Ile	Asp	Leu	Cys	Glu 85	Ser	Ser	Val	Gln	Arg 90	His	Leu	Gly	His	Ser 95	Asn
	Leu	Thr	Thr	Glu 100	Gln	Leu	Leu	Ala	Leu 105	Met	Glu	Ser	Leu	Pro 110	Gly	Lys
30	Lys	Ile	Ser 115	Pro	Glu	Glu	Ile	Glu 120	Arg	Thr	Arg	Lys	Thr 125	Cys	Lys	Ser
	Ser	Glu 130	Gln	Leu	Leu	Lys	Leu 135	Leu	Ser	Leu	Trp	Arg 140	Ile	Lys	Asn	Gly
35	Asp 145	Gln	Asp	Thr	Leu	Lys 150	Gly	Leu	Met	Tyr	Ala 155	Leu	Lys	His	Leu	Lys 160
40	Thr	Ser	His	Phe	Pro 165	Lys	Thr	Val	Thr	His 170	Ser	Leu	Arg	Lys	Thr 175	Met
	Arg	Phe	Leu	His 180	Ser	Phe	Thr	Met	Tyr 185	Arg	Leu	Tyr	Gln	Lys 190	Leu	Phe
45	Leu	Glu	Met 195	Ile	Gly	Asn	Gln	Val 200	Gln	Ser	Val	Lys	Ile 205	Ser	Cys	Leu
	(2) INFO	RMAT:	I NO	FOR S	SEQ I	ID NO	0:71	:	•							
50	(i)		LEI TYI	NGTH:	: 208	am: ac:	ino a id	acids	5							
55			тог	POLO	GY:	line	ar									

		(xi)	SEQU	JENCI	E DES	CRIE	MOIT	J: SE	EQ II	ON C	71:						
5		Lys 1	Суѕ	Gly	Ile	Asp 5	Val	Thr	Leu	Cys	Glu 10	Glu	Ala	Phe	Phe	Arg 15	Phe
Ū		Ala	Val	Pro	Thr 20	Lys	Phe	Thr	Pro	Asn 25	Trp	Leu	Ser	Val	Leu 30	Val	Asp
10		Asn	Leu	Pro 35	Gly	Thr	Lys	Val	Asn 40	Ala	Glu	Ser	Val	Glu 45	Arg	Ile	Lys
		Arg	Gln 50	His	Ser	Ser	Gln	Glu 55	Gln	Thr	Phe	Gln	Leu 60	Leu	Lys	Leu	Trp
15		Lys 65	His	Gln	Asn	Lys	Asp 70	Gln	Asp	Ile	Val	Lys 75	Lys	Ile	Ile	Gln	Asp 80
20		Ile	Asp	Leu	Cys	Glu 85	Asn	Ser	Val	Gln	Arg 90	His	Ile	Gly	His	Ala 95	Asn
		Leu	Thr	Phe	Glu 100	Gln	Leu	Arg	Ser	Leu 105	Met	Glu	Ser	Leu	Pro 110	Gly	Lys
25		Lys	Val	Gly 115	Ala	Glu	Asp	Ile	Glu 120	Lys	Thr	Ile	Lys	Ala 125	Cys	Lys	Pro
		Ser	Asp 130	Gln	Ile	Leu	Lys	Leu 135	Leu	Ser	Leu	Trp	Arg 140	Ile	Lys	Asn	Gly
30		Asp 145	Gln	Asp	Thr	Leu	Lys 150	Gly	Leu	Met	His	Ala 155	Leu	Lys	His	Ser	Lys 160
35		Thr	Tyr	His	Phe	Pro 165	Lys	Thr	Val	Thr	Gln 170	Ser	Leu	Lys	Lys	Thr 175	Ile
		Arg	Phe	Leu	His 180	Ser	Phe	Thr	Met	Tyr 185	Lys	Leu	Tyr	Gln	Lys 190	Leu	Phe
40		Leu	Glu	Met 195	Ile	Gly	Asn	Gln	Val 200	Gln	Ser	Val	Lys	Ile 205	Ser	Cys	Leu
	(2)	INFO	RMAT:	ION 1	FOR S	SEQ :	ID NO	0:72	:								
45		(i)	(A (B (C) LEI) TYI) STI	E CHANGTH PE: 1	: 483 nucle EDNES	B bas eic a SS: s	se pa acid sing:	airs								
50		(ii)			POLO												
55		(ix)	(A) NAI	: ME/KI CATI	EY: (CDS	83			-						
60		(xi)	SEQ	UENC	E DE	SCRI	PTIO	N: S	EQ I	D NO	:72:						
	GAT Asp 1	AGT (GTG ' Val (TGT (Cys :	CCC (Pro (5	CAA (Gln (GGA A	AAA ' Lys '	TAT . Tyr	ATC Ile 10	CAC His	CCT (Pro (CAA . Gln .	AAT A	AAT ' Asn : 15	TCG Ser	

5	ATT Ile	TGC Cys	TGT Cys	ACC Thr 20	AAG Lys	TGC Cys	CAC His	AAA Lys	GGA Gly 25	ACC Thr	TAC Tyr	TTG Leu	TAC Tyr	AAT Asn 30	GAC Asp	TGT Cys	96
-	CCA Pro	GGC Gly	CCG Pro 35	GGG Gly	CAG Gln	GAT Asp	ACG Thr	GAC Asp 40	TGC Cys	AGG Arg	GAG Glu	TGT Cys	GAG Glu 45	AGC Ser	GGC Gly	TCC Ser	144
10	TTC Phe	ACC Thr 50	GCT Ala	TCA Ser	GAA Glu	AAC Asn	CAC His 55	CTC Leu	AGA Arg	CAC His	TGC Cys	CTC Leu 60	AGC Ser	TGC Cys	TCC Ser	AAA Lys	192
15	TGC Cys 65	CGA Arg	AAG Lys	GAA Glu	ATG Met	GGT Gly 70	CAG Gln	GTG Val	GAG Glu	ATC Ile	TCT Ser 75	TCT Ser	TGC Cys	ACA Thr	GTG Val	GAC Asp 80	240
20	CGG Arg	GAC Asp	ACC Thr	GTG Val	TGT Cys 85	GGC Gly	TGC Cys	AGG Arg	AAG Lys	AAC Asn 90	CAG Gln	TAC Tyr	CGG Arg	CAT His	TAT Tyr 95	TGG Trp	288
25	AGT Ser	GAA Glu	AAC Asn	CTT Leu 100	TTC Phe	CAG Gln	TGC Cys	TTC Phe	AAT Asn 105	TGC Cys	AGC Ser	CTC Leu	TGC Cys	CTC Leu 110	AAT Asn	GGG Gly	336
	ACC Thr	GTG Val	CAC His 115	CTC Leu	TCC Ser	TGC Cys	CAG Gln	GAG Glu 120	AAA Lys	CAG Gln	AAC Asn	ACC Thr	GTG Val 125	TGC Cys	ACC Thr	TGC Cys	384
30	CAT His	GCA Ala 130	GGT Gly	TTC Phe	TTT Phe	CTA Leu	AGA Arg 135	GAA Glu	AAC Asn	GAG Glu	TGT Cys	GTC Val 140	TCC Ser	TGT Cys	AGT Ser	AAC Asn	432
35	TGT Cys 145	AAG Lys	AAA Lys	AGC Ser	CTG Leu	GAG Glu 150	TGC Cys	ACG Thr	AAG Lys	TTG Leu	TGC Cys 155	CTA Leu	CCC Pro	CAG Gln	ATT Ile	GAG Glu 160	480
40	AAT Asn																483
	(2)	INFO	ORMAT	rion	FOR	SEQ	ID N	10:73	3:								
45		((i) s	(B)	LEN TYI	CHAF IGTH: PE: &	161 minc	ami aci	no a	: acids	3						
50		(i	i) N	OLEC	ULE	TYPE	: pr	otei	n								
		(>	ci) S	SEQUE	ENCE	DESC	RIPT	ON:	SEC	O ID	NO:7	73:					
55	Asp 1	Ser	Val	Cys	Pro 5	Gln	Gly	Lys	Tyr	Ile 10	His	Pro	Gln	Asn	Asn 15	Ser	
	Ile	Cys	Cys	Thr 20	Lys	Cys	His	Lys	Gly 25	Thr	Tyr	Leu	Tyr	Asn 30	Asp	Cys	
60	Pro	Gly	Pro 35	Gly	Gln	qzA	Thr	Asp 40	Cys	Arg	Glu	Cys	Glu 45	Ser	Gly	Ser	

	Pne	50	Ala	ser	GIU	Asn	H1S 55	Leu	Arg	His	Cys	Leu 60	Ser	Cys	Ser	Lys		
5	Cys 65	Arg	Lys	Glu	Met	Gly 70	Gln	Val	Glu	Ile	Ser 75	Ser	Cys	Thr	Val	qaA 08		
	Arg	Asp	Thr	Val	Cys 85	Gly	Cys	Arg	Lys	Asn 90	Gln	Tyr	Arg	His	Tyr 95	Trp		
LO	Ser	Glu	Asn	Leu 100	Phe	Gln	Cys	Phe	Asn 105	Cys	Ser	Leu	Cys	Leu 110	Asn	Gly		
L5	Thr	Val	His 115	Leu	Ser	Cys	Gln	Glu 120	Lys	Gln	Asn	Thr	Val 125	Cys	Thr	Cys		
	His	Ala 130	Gly	Phe	Phe	Leu	Arg 135	Glu	Asn	Glu	Cys	Val 140	Ser	Cys	Ser	Asn		
20	Cys 145	Lys	Lys	Ser	Leu	Glu 150	Cys	Thr	Lys	Leu	Cys 155	Leu	Pro	Gln	Ile	Glu 160		
	Asn																	
25	(2)			rion		_												
30		(1)	() () ()	QUENC A) LI B) T' C) S' C) T'	ENGTI PE: PRANI	H: 70 nuc: DEDNI	05 ba leic ESS:	ase p acid	pairs 1	5								
2.5		(ii)	MOI	LECUI	E T	YPE:	CDN	A										
35		(ix	()	ATURI A) NA B) LO	AME/			705										
40		(xi) SE	QUEN	CE D	ESCR:	IPTI	ON:	SEQ :	ID N	0:74	:						
45	TTG Leu 1	CCC Pro	GCC Ala	CAG Gln	GTG Val 5	GCA Ala	TTT Phe	ACA Thr	CCC Pro	TAC Tyr 10	GCC Ala	CCG Pro	GAG Glu	CCC Pro	GGG Gly 15	AGC Ser	48	
EΛ	ACA Thr	TGC Cys	CGG Arg	CTC Leu 20	AGA Arg	GAA Glu	TAC Tyr	TAT Tyr	GAC Asp 25	CAG Gln	ACA Thr	GCT Ala	CAG Gln	ATG Met 30	TGC Cys	TGC Cys	96	
50	AGC Ser	AAG Lys	TGC Cys 35	TCG Ser	CCG Pro	GGC Gly	CAA Gln	CAT His 40	GCA Ala	AAA Lys	GTC Val	TTC Phe	TGT Cys 45	ACC Thr	AAG Lys	ACC Thr	144	
55	TCG Ser	GAC Asp 50	ACC Thr	GTG Val	TGT Cys	GAC Asp	TCC Ser 55	TGT Cys	GAG Glu	GAC Asp	AGC Ser	ACA Thr 60	Tyr	ACC Thr	CAG Gln	CTC Leu	192	
60	TGG Trp 65	Asn	TGG Trp	GTT Val	CCC Pro	GAG Glu 70	TGC Cys	TTG Leu	AGC Ser	TGT Cys	GGC Gly 75	Ser	CGC Arg	TGT Cys	AGC Ser	TCT Ser 80	240	

	GAC Asp	CAG Gln	GTG Val	GAA Glu	ACT Thr 85	CAA Gln	GCC Ala	TGC Cys	ACT Thr	CGG Arg 90	GAA Glu	CAG Gln	AAC Asn	CGC Arg	ATC Ile 95	TGC Cys	288
5	ACC Thr	TGC Cys	AGG Arg	CCC Pro 100	GGC Gly	TGG Trp	TAC Tyr	TGC Cys	GCG Ala 105	CTG Leu	AGC Ser	AAG Lys	CAG Gln	GAG Glu 110	GGG Gly	TGC Cys	336
10	CGG Arg	CTG Leu	TGC Cys 115	GCG Ala	CCG Pro	CTG Leu	CGC Arg	AAG Lys 120	TGC Cys	CGC Arg	CCG Pro	GGC Gly	TTC Phe 125	GGC Gly	GTG Val	GCC Ala	384
15	AGA Arg	CCA Pro 130	GGA Gly	ACT Thr	GAA Glu	ACA Thr	TCA Ser 135	GAC Asp	GTG Val	GTG Val	TGC Cys	AAG Lys 140	CCC Pro	TGT Cys	GCC Ala	CCG Pro	432
20	GGG Gly 145	ACG Thr	TTC Phe	TCC Ser	AAC Asn	ACG Thr 150	ACT Thr	TCA Ser	TCC Ser	ACG Thr	GAT Asp 155	ATT Ile	TGC Cys	AGG Arg	CCC Pro	CAC His 160	480
_ `	CAG Gln	ATC Ile	TGT Cys	AAC Asn	GTG Val 165	GTG Val	GCC Ala	ATC Ile	CCT Pro	GGG Gly 170	AAT Asn	GCA Ala	AGC Ser	AGG Arg	GAT Asp 175	GCA Ala	528
25	GTC Val	TGC Cys	ACG Thr	TCC Ser 180	ACG Thr	TCC Ser	CCC Pro	ACC Thr	CGG Arg 185	AGT Ser	ATG Met	GCC Ala	CCA Pro	GGG Gly 190	GCA Ala	GTA Val	576
30	CAC His	TTA Leu	CCC Pro 195	CAG Gln	CCA Pro	GTG Val	TCC Ser	ACA Thr 200	CGA Arg	TCC Ser	CAA Gln	CAC His	ACG Thr 205	CAG Gln	CCA Pro	ACT Thr	624
35	CCA Pro	GAA Glu 210	CCC Pro	AGC Ser	ACT Thr	GCT Ala	CCA Pro 215	AGC Ser	ACC Thr	TCC Ser	TTC Phe	CTG Leu 220	CTC Leu	CCA Pro	ATG Met	GGC	672
40	CCC Pro 225	AGC Ser	CCC Pro	CCA Pro	GCT Ala	GAA Glu 230	GGG Gly	AGC Ser	ACT Thr	GGC Gly	GAC Asp 235						705
	(2)	INFO	ORMA'	rion	FOR	SEQ	ID 1	10:7	5:								
45	•	,	(i) S	(B)	LEI	VGTH:	235 mino	am:	ino a id	: acids	5						
50				MOLE SEQUI						חד כ	NO.	75.					
	Leu			Gln	Val								Glu	Pro	Gly	Ser	
55	Thr	Cve	λνα	Lon	5	C1		(Th	3	10	m 1		.		15	_	
	1111	Суз	Arg	Leu 20	AIG	GIU	туг	туг	Asp 25	GIN	Tnr	Ala	GIN	30	Cys	Cys	
60	Ser	Lys	Cys 35	Ser	Pro	Gly	Gln	His 40	Ala	Lys	Val	Phe	Cys 45	Thr	Lys	Thr	
	Ser	Asp 50	Thr	Val	Cys	Asp	Ser 55	Суѕ	Glu	Asp	Ser	Thr	Tyr	Thr	Gln	Leu	

	65	Asn	Trp	vaı	Pro	70	Cys	Leu	Ser	Cys	75	Ser	Arg	Cys	Ser	Ser 80	
5	Asp	Gln	Val	Glu	Thr 85	Gln	Ala	Cys	Thr	Arg 90	Glu	Gln	Asn	Arg	Ile 95	Cys	
10	Thr	Cys	Arg	Pro 100	Gly	Trp	Tyr	Cys	Ala 105	Leu	Ser	Lys	Gln	Glu 110	Gly	Cys	
	Arg	Leu	Cys 115	Ala	Pro	Leu	Arg	Lys 120	Cys	Arg	Pro	Gly	Phe 125	Gly	Val	Ala	
15	Arg	Pro 130	Gly	Thr	Glu	Thr	Ser 135	Asp	Val	Val	Суѕ	Lys 140	Pro	Cys	Ala	Pro	
	Gly 145	Thr	Phe	Ser	Asn	Thr 150	Thr	Ser	Ser	Thr	Asp 155	Ile	Cys	Arg	Pro	His 160	
20	Gln	Ile	Cys	Asn	Val 165	Val	Ala	Ile	Pro	Gly 170	Asn	Ala	Ser	Arg	Asp 175	Ala	
25	Val	Cys	Thr	Ser 180	Thr	Ser	Pro	Thr	Arg 185	Ser	Met	Ala	Pro	Gly 190	Ala	Val	
 .	His	Leu	Pro 195	Gln	Pro	Val	Ser	Thr 200	Arg	Ser	Gln	His	Thr 205	Gln	Pro	Thr	
30	Pro	Glu 210	Pro	Ser	Thr	Ala	Pro 215	Ser	Thr	Ser	Phe	Leu 220	Leu	Pro	Met	Gly	
	Pro 225	Ser	Pro	Pro	Ala	Glu 230	Gly	Ser	Thr	Gly	Asp 235						
35	(2)	INF	ORMA!	rion	FOR	SEQ	ID I	NO : 7	6 :								
4 0		(i)	(1 (1	c) s:		H: 4: ami: DEDN:	20 ai no a ESS:	mino cid sin	aci	ds							
45		(ii)) MOI	LECUI	LE T	YPE:	pro	tein							·		
 .																	
		(xi)) SE	QUEN	CE DI	ESCR:	IPTI	: NC	SEQ	ID N	0:76	:					,
50		Met 1	t Gly	y Lei	ı Se	r Th	r Va	l Pr	o As	p Le	u Le 10	u Le	u Pr	o Le	u Va	l Leu 15	Leu
55		Gli	ı Lei	u Lei	u Va: 20	l Gl	y Il	е Ту	r Pr	o Se 25		y Va	l Il	e Gl	y Le	u Val	Pro
J J		His	s Le	u Gly 35	y Ası	p Ar	g Gl	u Ly	s Ar 40	g As	p Se	r Va	l Cy	s Pr	o Gl	n Gly	Lys
60		ТУ	r Ile 50	e Hi	s Pro	o G1:	n As:	n As: 55	n Se	r Il	е Су	s Cy	s Th 60	r Ly	s Cy	s His	Lys
		Gl ₃ 65	y Th:	r Ty:	r Lei	и Ту	r As:	n As	р Су	s Pr	o Gl	y Pr	o Gl	y Gl	n As	p Thr	Asr 80

	Cys	Arg	Glu	Суз	Glu 85	Ser	Gly	Ser	Phe	Thr 90	Ala	Ser	Glu	Asn	His 95	Leu
5	Arg	His	Cys	Leu 100	Ser	Cys	Ser	Lys	Cys 105	Arg	Lys	Glu	Met	Gly 110	Gln	Val
10	Glu	Ile	Ser 115	Ser	Cys	Thr	Val	Asp 120	Arg	Asp	Thr	Val	Cys 125	Gly	Cys	Arg
10	Lys	Asn 130	Gln	Tyr	Arg	His	Tyr 135	Trp	Ser	Glu	Asn	Leu 140	Phe	Gln	Cys	Phe
15	Asn 145	Cys	Ser	Leu	Cys	Leu 150	Asn	Gly	Thr	Val	His 155	Leu	Ser	Cys	Gln	Glu 160
	Lys	Gln	Asn	Thr	Val 165	Cys	Thr	Cys	His	Ala 170	Gly	Phe	Phe	Leu	Arg 175	Glu
20	Asn	Glu	Cys	Val 180	Ser	Cys	Ser	Asn	Cys 185	Lys	Lys	Ser	Leu	Glu 190	Cys	Thr
25	Lys	Leu	Cys 195	Leu	Pro	Gln	Ile	Glu 200	Asn	Val	Lys	Gly	Thr 205	Glu	Asp	Ser
	Gly	Thr 210	Thr	Gly	Lys	Cys	Gly 215	Ile	Asp	Val	Thr	Leu 220	Cys	Glu	Glu	Ala
30	Phe 225	Phe	Arg	Phe	Ala	Val 230	Pro	Thr	Lys	Phe	Thr 235	Pro	Asn	Trp	Leu	Ser 240
	Val	Leu	Val	Asp	Asn 245	Leu	Pro	Gly	Thr	Lys 250	Val	Asn	Ala	Glu	Ser 255	Val
35	Glu	Arg	Ile	Lys 260	Arg	Gln	His	Ser	Ser 265	Gln	Glu	Gln	Thr	Phe 270	Gln	Leu
40	Leu	Lys	Leu 275	Trp	Lys	His	Gln	Asn 280	Lys	Asp	Gln	Asp	Ile 285	Val	Lys	Lys
	Ile	Ile 290	Gln	Asp	Ile	Asp	Leu 295	Cys	Glu	Asn	Ser	Val 300	Gln	Arg	His	Ile
45	Gly 305	His	Ala	Asn	Leu	Thr 310	Phe	Glu	Gln	Leu	Arg 315	Ser	Leu	Met	Glu	Ser 320
	Leu	Pro	Gly	Lys	Lys 325	Val	Gly	Ala	Glu	Asp 330	Ile	Glu	Lys	Thr	Ile 335	Lys
50	Ala	Cys	Lys	Pro 340	Ser	Asp	Gln	Ile	Leu 345	Lys	Leu	Leu	Ser	Leu 350	Trp	Arg
55	Ile	Lys	Asn 355	Gly	Asp	Gln	Asp	Thr 360	Leu	Lys	Gly	Leu	Met 365	His	Ala	Leu
	Lys	His 370	Ser	Lys	Thr	Tyr	His 375	Phe	Pro	Lys	Thr	Val 380	Thr	Gln	Ser	Leu
60	Lys 385	Lys	Thr	Ile	Arg	Phe 390	Leu	His	Ser	Phe	Thr 395	Met	Tyr	Lys	Leu	Туг 400
	Gln	Lys	Leu	Phe	Leu 405	Glu	Met	Ile	Gly	Asn 410	Gln	Val	Gln	Ser	Val 415	Lys

Ile Ser Cys Leu 420

5	(2)	INFO	RMAT	ON I	FOR S	SEQ I	D NO):77:	:								
10		(i)	(B) (C)	LEI TYI	GTH: PE: 6 RANDI	: 211 amino EDNES	Lami caci SS: s	ino a id sing]	acids	5							
15		(ii)	MOLI	ECULI	E TYI	PE: I	orote	ein									
		(xi)	SEQ	JENCI	E DES	SCRII	OITS	1: SI	EQ II	NO:	:77:						
20		Met 1	Gly	Leu	Ser	Thr 5	Val	Pro	Asp	Leu	Leu 10	Leu	Pro	Leu	Val	Leu 15	Leu
25		Glu	Leu	Leu	Val 20	Gly	Ile	Tyr	Pro	Ser 25	Gly	Val	Ile	Gly	Leu 30	Val	Pro
23		His	Leu	Gly 35	Asp	Arg	Glu	Lys	Arg 40	Asp	Ser	Val	Cys	Pro 45	Gln	Gly	Lys
30		Tyr	Ile 50	His	Pro	Gln	Asn	Asn 55	Ser	Ile	Cys	Cys	Thr 60	Lys	Cys	His	Lys
		Gly 65	Thr	Tyr	Leu	Tyr	Asn 70	Asp	Cys	Pro	Gly	Pro 75	Gly	Gln	Asp	Thr	Asp 80
35		Cys	Arg	Glu	Cys	Glu 85	Ser	Gly	Ser	Phe	Thr 90	Ala	Ser	Glu	Asn	His 95	Leu
40		Arg	His	Cys	Leu 100	Ser	Суѕ	Ser	Lys	Cys 105	Arg	Lys	Glu	Met	Gly 110	Gln	Val
		Glu	Ile	Ser 115	Ser	Cys	Thr	Val	Asp 120	Arg	Asp	Thr	Val	Cys 125	Gly	Cys	Arg
45		Lys	Asn 130	Gln	Tyr	Arg	His	Tyr 135	Trp	Ser	Glu	Asņ	Leu 140	Phe	Gln	Cys	Phe
		Asn 145	Суѕ	Ser	Leu	Суз	Leu 150	Asn	Gly	Thr	Val	His 155	Leu	Ser	Cys	Gln	Glu 160
50		Lys	Gln	Asn	Thr	Val 165	Суѕ	Thr	Cys	His	Ala 170	Gly	Phe	Phe	Leu	Arg 175	Glu
55		Asn	Glu	Cys	Val 180	Ser	Cys	Ser	Asn	Cys 185	Lys	Lys	Ser	Leu	Glu 190	Cys	Thr
		Lys	Leu	Cys 195	Leu	Pro	Gln	Ile	Glu 200	Asn	Val	Lys	Gly	Thr 205	Glu	Asp	Ser
60		Gly	Thr 210	Thr													

(2)	INFORMATION	FOR	SEQ	ID	NO:78:
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5	(i)	(A) (B) (C)	LEI TYI STI	NGTH PE: 6 RANDI	ARACT : 417 amind EDNES GY: 1	7 ami o aci SS: s	ino a id sing:	acids	5							
10	(ii)	MOLI	ECULI	E TYI	PE: p	prote	ein					•				
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20	Glu	Leu	Leu	Val 20	Gly	Ile	Tyr	Pro	Ser 25	Gly	Val	Ile	Gly	Leu 30	Val	Pro
	His	Leu	Gly 35	Asp	Arg	Glu	Lys	Arg 40	Asp	Ser	Val	Cys	Pro 45	Gln	Gly	ГУS
25	Tyr	Ile 50	His	Pro	Gln	Asn	Asn 55	Ser	Ile	Cys	Cys	Thr 60	Lys	Cys	His	Lys
30	Gly 65	Thr	Tyr	Leu	Tyr	Asn 70	Asp	Cys	Pro	Gly	Pro 75	Gly	Gln	Asp	Thr	Asp 80
30	Суз	Arg	Glu	Cys	Glu 85	Ser	Gly	Ser	Phe	Thr 90	Ala	Ser	Glu	Asn	His 95	Leu
35	Arg	His	Сув	Leu 100	Ser	Cys	Ser	Lys	Cys 105	Arg	Lys	Glu	Met	Gly 110	Gln	Va]
	Glu	Ile	Ser 115	Ser	Суѕ	Thr	Val	Asp 120	Arg	Asp	Thr	Val	Cys 125	Gly	Cys	Arg
40	Lys	Asn 130	Gln	Tyr	Arg	His	Tyr 135	Trp	Ser	Glu	Asn	Leu 140	Phe	Gln	Cys	Phe
4 =	Asn 145	Cys	Ser	Leu	Cys	Leu 150	Asn	Gly	Thr	Val	His 155	Leu	Ser	Cys	Gln	Glu 160
45	Lys	Gln	Asn	Thr	Val 165	Cys	Thr	Cys	His	Ala 170	Gly	Phe	Phe	Leu	Arg 175	Glu
50	Asn	Glu	Cys	Val 180	Ser	Cys	Ser	Asn	Cys 185	Lys	Lys	Ser	Leu	Glu 190	Cys	Thi
	Lys	Leu	Cys 195	Leu	Pro	Gln	Ile	Glu 200	Asn	Val	Lys	Gly	Thr 205	Glu	Asp	Sei
55	Gly	Thr 210	Thr	Gly	Ile	Asp	Val 215	Thr	Leu	Cys	Glu	Glu 220	Ala	Phe	Phe	Arg
60	Phe 225	Ala	Val	Pro	Thr	Lys 230	Phe	Thr	Pro	Asn	Trp 235	Leu	Ser	Val	Leu	Va:
60	Asp	Asn	Leu	Pro	Gly 245	Thr	Lys	Val	Asn	Ala 250	Glu	Ser	Val	Glu	Arg 255	

	Lys	Arg	Gln	His 260	Ser	Ser	Gln	Glu	Gln 265	Thr	Phe	Gln	Leu	Leu 270	Lys	Leu
5	Trp	Lys	His 275	Gln	Asn	Lys	Asp	Gln 280	Asp	Ile	Val	Lys	Lys 285	Ile	Ile	Gln
	Asp	Ile 290	Asp	Leu	Cys	Glu	Asn 295	Ser	Val	Gln	Arg	His 300	Ile	Gly	His	Ala
10	Asn 305	Leu	Thr	Phe	Glu	Gln 310	Leu	Arg	Ser	Leu	Met 315	Glu	Ser	Leu	Pro	Gly 320
15	Lys	Lys	Val	Gly	Ala 325	Glu	Asp	Ile	Glu	Lys 330	Thr	Ile	Lys	Ala	Cys 335	Lys
	Pro	Ser	Asp	Gln 340	Ile	Leu	Lys	Leu	Leu 345	Ser	Leu	Trp	Arg	Ile 350	Lys	Asn
20	Gly	Asp	Gln 355	Asp	Thr	Leu	Lys	Gly 360	Leu	Met	His	Ala	Leu 365	Lys	His	Ser
	Lys	Thr 370	Tyr	His	Phe	Pro	Lys 375	Thr	Val	Thr	Gln	Ser 380	Leu	Lys	Lys	Thr
25	Ile 385	Arg	Phe	Ļeu	His	Ser 390	Phe	Thr	Met	Tyr	Lys 395	Leu	Tyr	Gln	Lys	Leu 400
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	Leu															
35	(2) INFO															
40	(i)	(B)	LEI TYI STI	NGTH PE: 8 RANDI	: 397 amino EDNES	7 am: o ac: SS: s	ino a id sing:	acids	3							
40	(ii)	MOLI		POLOG												
45																
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	Glu	Leu	Leu	Val 20	Gly	Ile	Tyr	Pro	Ser 25	Gly	Val	Ile	Gly	Leu 30	Val	Pro
55	His	Leu	Gly 35	Asp	Arg	Glu	Lys	Arg 40	Asp	Ser	Val	Cys	Pro 45	Gln	Gly	Lys
60	Tyr	Ile 50	His	Pro	Gln	Asn	Asn 55	Ser	Ile	Cys	Cys	Thr 60	Lys	Cys	His	Lys
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	Cys	Arg	Glu	Cys	Glu 85	Ser	Gly	Ser	Phe	Thr 90	Ala	Ser	Glu	Asn	His 95	Leu
5	Arg	His	Cys	Leu 100	Ser	Cys	Ser	Lys	Cys 105	Arg	Lys	Glu	Met	Gly 110	Gln	Val
	Glu	Ile	Ser 115	Ser	Cys	Thr	Val	Asp 120	Arg	Asp	Thr	Val	Cys 125	Gly	Cys	Arg
10	Lys	Asn 130	Gln	Tyr	Arg	His	Tyr 135	Trp	Ser	Glu	Asn	Leu 140	Phe	Gln	Cys	Phe
15	Asn 145	Cys	Ser	Leu	Cys	Leu 150	Asn	Gly	Thr	Val	His 155	Leu	Ser	Cys	Gln	Glu 160
	Lys	Gln	Asn	Thr	Val 165	Cys	Thr	Cys	His	Ala 170	Gly	Phe	Phe	Leu	Arg 175	Gļu
20	Asn	Glu	Cys	Val 180	Ser	Cys	Ser	Asn	Cys 185	Lys	Lys	Ser	Leu	Glu 190	Cys	Thr
	Lys	Leu	Cys 195	Leu	Pro	Gln	Ile	Glu 200	Asn	Val	Lys	Gly	Thr 205	Glu	Asp	Ser
25	Gly	Thr 210	Thr	Gly	Pro	Asn	Trp 215	Leu	Ser	Val	Leu	Val 220	Asp	Asn	Leu	Pro
30	Gly 225	Thr	Lys	Val	Asn	Ala 230	Glu	Ser	Val	Glu	Arg 235	Ile	Lys	Arg	Gln	His 240
	Ser	Ser	Gln	Glu	Gln 245	Thr	Phe	Gln	Leu	Leu 250	Lys	Leu	Trp	Lys	His 255	Gln
35	Asn	Lys	Asp	Gln 260	Asp	Ile	Val	Lys	Lys 265	Ile	Ile	Gln	Asp	Ile 270	Asp	Leu
	Cys	Glu	Asn 275	Ser	Val	Gln	Arg	His 280	Ile	Gly	His	Ala	Asn 285	Leu	Thr	Phe
40	Glu	Gln 290	Leu	Arg	Ser	Leu	Met 295	Glu	Ser	Leu	Pro	Gly 300	Lys	Lys	Val	Gly
45	Ala 305	Glu	Asp	Ile	Glu	Lys 310	Thr	Ile	Lys	Ala	Cys 315	Lys	Pro	Ser	Asp	Gln 320
	Ile	Leu	Lys	Leu	Leu 325	Ser	Leu	Trp	Arg	Ile 330	Lys	Asn	Gly	Asp	Gln 335	Asp
50	Thr	Leu	Lys	Gly 340	Leu	Met	His	Ala	Leu 345	Lys	His	Ser	Lys	Thr 350	Tyr	His
	Phe	Pro	Lys 355	Thr	Val	Thr	Gln	Ser 360	Leu	Lys	Lys	Thr	Ile 365	Arg	Phe	Leu
55	His	Ser 370	Phe	Thr	Met	Tyr	Lys 375	Leu	Tyr	Gln	Lys	Leu 380	Phe	Leu	Glu	Met
60	Ile 385	Gly	Asn	Gln	Val	Gln 390	Ser	Val	Lys	Ile	Ser 395	Суз	Leu			

(2)	INFORMATION	FOR	SEO	TD	NO. SO.
\~/	THE OTHER POIN	LOIC	Sug	TD	MO:OU:

5	(i)	SEQU (A) (B) (C) (D)	LEN TYP STF	IGTH: PE: & RANDI	ARACT 366 amino EDNES SY: 1	ami aci SS: s	no a d singl	acids	3							
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15	(xi)	SEQU	JENCE	E DES	SCRIE	OITS	1: SE	EQ II	NO:	:08						
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20	Glu	Leu	Leu	Val 20	Gly	Ile	Tyr	Pro	Ser 25	Gly	Val	Ile	Gly	Leu 30	Val	Pro
	His	Leu	Gly 35	Asp	Arg	Glu	Lys	Arg 40	Asp	Ser	Val	Суѕ	Pro 45	Gln	Gly	Lys
25	Tyr	Ile 50	His	Pro	Gln	Asn	Asn 55	Ser	Ile	Cys	Cys	Thr 60	Lys	Cys	His	Lys
30	Gly 65	Thr	Tyr	Leu	Tyr	Asn 70	Asp	Cys	Pro	Gly	Pro 75	Gly	Gln	Asp	Thr	Asp 80
30	Cys	Arg	Glu	Cys	Glu 85	Ser	Gly	Ser	Phe	Thr 90	Ala	Ser	Glu	Asn	His 95	Leu
35	Arg	His	Cys	Leu 100	Ser	Cys	Ser	Lys	Cys 105	Arg	Lys	Glu	Met	Gly 110	Gln	Va]
	Glu	Ile	Ser 115	Ser	Суѕ	Thr	Val	Asp 120	Arg	Asp	Thr	Val	Cys 125	Gly	Cys	Arg
40	Lys	Asn 130	Gln	Tyr	Arg	His	Tyr 135	Trp	Ser	Glu	Asn	Leu 140	Phe	Gln	Cys	Phe
45	Asn 145	Суѕ	Ser	Leu	Суз	Leu 150	Asn	Gly	Thr	Val	His 155	Leu	Ser	Cys	Gln	Glu 160
40	Lys	Gln	Asn	Thr	Val 165	Суѕ	Thr	Суѕ	His	Ala 170	Gly	Phe	Phe	Leu	Arg 175	Glı
50	Asn	Glu	Суѕ	Val 180	Ser	Cys	Ser	Asn	Cys 185	Lys	Lys	Ser	Leu	Glu 190	Cys	Thi
	Lys	Leu	Cys 195	Leu	Pro	Gln	Ile	Glu 200	Asn	Val	Lys	Gly	Thr 205	Glu	Asp	Sei
55	Gly	Thr 210	Thr	Gly	Glu	Gln	Thr 215	Phe	Gln	Leu	Leu	Lys 220	Leu	Trp	Lys	His
60	Gln 225	Asn	Lys	Asp	Gln	Asp 230	Ile	Val	Lys	Lys	Ile 235	Ile	Gln	Asp	Ile	Asp 240
60	Leu	Суѕ	Glu	Asn	Ser 245	Val	Gln	Arg	His	Ile 250	Gly	His	Ala	Asn	Leu 255	Th

	Phe	Glu	Gln	Leu 260	Arg	Ser	Leu	Met	Glu 265	Ser	Leu	Pro	Gly	Lys 270	Lys	Val
5	Gly	Ala	Glu 275	Asp	Iļe	Glu	Lys	Thr 280	Ile	Lys	Ala	Cys	Lys 285	Pro	Ser	Asp
	Gln	Ile 290	Leu	Lys	Leu	Leu	Ser 295	Leu	Trp	Arg	Ile	Lys 300	Asn	Gly	Asp	Gln
10	Asp 305	Thr	Leu	Lys	Gly	Leu 310	Met	His	Ala	Leu	Lys 315	His	Ser	Lys	Thr	Туг 320
15	His	Phe	Pro	Lys	Thr 325	Val	Thr	Gln	Ser	Leu 330	Lys	Lys	Thr	Ile	Arg 335	Phe
	Leu	His	Ser	Phe 340	Thr	Met	Tyr	Lys	Leu 345	Tyr	Gln	Lys	Leu	Phe 350	Leu	Glu
20	Met	Ile	Gly 355	Asn	Gln	Val	Gln	Ser 360	Val	Lys	Ile	Ser	Cys 365	Leu		
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25	(i)	(B)	LEI TYI	NGTH: PE: & RANDI	: 31: amino EDNES	Lam: cac: SS: s	ino a id singl	acids	5							
30	(ii)	MOLE	ECULI	Е ТҮІ	PE: p	prote	ein									
35	(xi)															
4.0	1	Gly			5					10					15	
40		Leu		20					25					30		
45		Leu	35					40					45			
		Ile 50					55					60				
50	Gly 65	Thr	Туr	Leu	Tyr	Asn 70	Asp	Cys	Pro	Gly	Pro 75	Gly	Gln	Asp	Thr	Asp 80
	Cys	Arg	Glu	Cys	Glu 85	Ser	Gly	Ser	Phe	Thr 90	Ala	Ser	Glu	Asn	His 95	Leu
55	Arg	His	Суѕ	Leu 100	Ser	Суѕ	Ser	Lys	Cys 105	Arg	Lys	Glu	Met	Gly 110	Gln	Val
60	Glu	Ile	Ser 115	Ser	Суѕ	Thr	Val	Asp 120	Arg	Asp	Thr	Val	Cys 125	Gly	Cys	Arg
	Lys	Asn 130	Gln	Tyr	Arg	His	Tyr 135	Trp	Ser	Glu	Asn	Leu 140	Phe	Gln	Суѕ	Phe

	145	Cys	Ser	Leu	Cys	Leu 150	Asn	Gly	Thr	Val	His 155	Leu	Ser	Cys	Gln	Glu 160
5	Lys	Gln	Asn	Thr	Val 165	Cys	Thr	Cys	His	Ala 170	Gly	Phe	Phe	Leu	Arg 175	
	Asn	Glu	Cys	Val 180	Ser	Cys	Ser	Asn	Cys 185	Lys	Lys	Ser	Leu	Glu 190	Cys	Thr
10.	Lys	Leu	Cys 195	Leu	Pro	Gln	Ile	Glu 200	Asn	Val	Lys	Gly	Thr 205	Glu	Asp	Ser
15	Gly	Thr 210	Thr	Gly	Pro	Gly	Lys 215	Lys	Val	Gly	Ala	Glu 220	Asp	Ile	Glu	Lys
	Thr 225	Ile	Lys	Ala	Cys	Lys 230	Pro	Ser	Asp	Gln	Ile 235	Leu	Lys	Leu	Leu	Ser 240
20	Leu	Trp	Arg	Ile	Lys 245	Asn	Gly	Asp	Gln	Asp 250	Thr	Leu	Lys	Gly	Leu 255	Met
	His	Ala	Leu	Lys 260	His	Ser	Lys	Thr	Tyr 265	His	Phe	Pro	Lys	Thr 270	Val	Thr
25	Gln	Ser	Leu 275	Lys	Lys	Thr	Ile	Arg 280	Phe	Leu	His	Ser	Phe 285	Thr	Met	Tyr
30	Lys	Leu 290	Tyr	Gln	Lys	Leu	Phe 295	Leu	Glu	Met	Ile	Gly 300	Asn	Gln	Val	Gln
30	Ser 305	Val	Lys	Ile	Ser	Cys 310	Leu									
35	(2) INFO	RMAT:	ION I	FOR S	SEQ :	ID NO	0:82	:								
40	(i)	(B)) LEI) TYI) STI	NGTH PE: 3 RANDI	: 100 amino EDNE:	6 am: o ac: SS: s	ino a id singl	acids	5							
40	(ii)	MOĖ!				linea prote										
45					•											
	(xi)	SEQ	UENCI	E DE	SCRI	PTIO	N: SI	EQ II	о ио	82:						
50	Met 1	Asp	Ser	Val	Cys 5	Pro	Gln	Gly	Lys	Tyr 10	Ile	His	Pro	Gln	Asn 15	Asn
	Ser	Ile	Cys	Cys 20	Thr	Lys	Суз	His	Lys 25	Gly	Thr	Tyr	Leu	Tyr 30	Asn	Asp
55	Cys	Pro	Gly 35	Pro	Gly	Gln	Asp	Thr 40	Asp	Суз	Arg	Glu	Cys 45	Glu	Ser	Gly
60	Ser	Phe 50	Thr	Ala	Ser	Glu	Asn 55	His	Leu	Arg	His	Суs 60	Leu	Ser	Суз	Ser
	Lys 65	Суѕ	Arg	Lys	Glu	Met 70	Gly	Gln	Val	Glu	Ile 75	Ser	Ser	Суѕ	Thr	Val 80

	Asp	Arg	Asp	Thr	Val 85	Суз	Gly	Cys	Arg	Lys 90	Asn	Gln	Tyr	Arg	His 95	Tyr
5	Trp	Ser	Glu	Asn 100	Leu	Phe	Gln	Cys	Phe 105	Cys						
	(2) INFO	RMAT:	ION I	FOR S	SEQ I	ID NO	0:83	:								
10	(i)	SEQUAL (A)) LEI) TYI) STI	IGTH: PE: & RANDI	: 109 amino EDNES	reris 9 ami 5 aci 5S: s	ino a id sing]	acids	5							
15	(ii)	MOL	ECULI	E TYI	?E: 1	prote	ein									
20	(xi)	SEQ	JENCI	E DES	SCRII	PTIOI	1: SI	EQ II	оио:	:83:						
	Met 1	Asp	Ser	Val	Суs 5	Pro	Gln	Gly	Lys	Tyr 10	Ile	His	Pro	Gln	Asn 15	Asn
25	Ser	Ile	Cys	Cys 20	Thr	Lys	Cys	His	Lys 25	Gly	Thr	Tyr	Leu	Tyr 30	Asn	Asp
30	Cys	Pro	Gly 35	Pro	Gly	Gln	Asp	Thr 40	Asp	Cys	Arg	Glu	Cys 45	Glu	Ser	Gly
	Ser	Phe 50	Thr	Ala	Ser	Glu	Asn 55	His	Leu	Arg	His	Суs 60	Leu	Ser	Cys	Ser
35	Lys 65	Cys	Arg	Lys	Glu	Met 70	Gly	Gln	Val	Glu	Ile 75	Ser	Ser	Суѕ	Thr	Val 80
	Asp	Arg	Asp	Thr	Val 85	Cys	Gly	Суѕ	Arg	Lys 90	Asn	Gln	Tyr	Arg	His 95	Tyr
40	Trp	Ser	Glu	Asn 100	Leu	Phe	Gln	Cys	Phe 105	Asn	Cys	Ser	Leu		•	
	(2) INFO	RMAT:	ION F	FOR S	SEQ :	ID NO	0:84	•								
45	(i)	(A) (B) (C)) STI	NGTH: PE: & RANDI	: 109 amino EDNES	9 am: 5 ac: 5S: 5	ino a id sing:	acida	3							
50	(ii)	MOLI				linea prote										
55																
	(xi)	SEQ	JENCI	E DES	SCRI	PTIO	N: S1	EQ I	ои о	:84:						
60	Met 1	Asp	Ser	Val	Cys 5	Pro	Gln	Gly	Lys	Tyr 10	Ile	His	Pro	Gln	Asn 15	Asn
~ ~	Ser	Ile	Суѕ	Cys 20	Thr	Lys	Cys	His	Lys 25	Gly	Thr	Tyr	Leu	Tyr 30	Asn	Asp

		Cys	Pro	Gly 35	Pro	Gly	Gln	Asp	Thr 40	Asp	Cys	Arg	Glu	Cys 45	Glu	Ser	Gly
5		Ser	Phe 50	Thr	Ala	Ser	Glu	Asn 55	His	Leu	Arg	His	Cys 60	Leu	Ser	Cys	Ser
		Lys 65	Суз	Arg	Lys	Glu	Met 70	Gly	Gln	Val	Glu	Ile 75	Ser	Ser	Cys	Thr	Val 80
10		Asp	Arg	Asp	Thr	Val 85	Cys	Gly	Cys	Arg	Lys 90	Asn	Gln	Tyr	Arg	His 95	Tyr
15		Trp	Ser	Glu	Asn 100	Leu	Phe	Gln	Cys	Phe 105	Asn	Cys	Ser	Leu			
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20		(i)	SEQUAL (A)) STI	VGTH:	: 101 amino EDNES	l ami o aci SS: s	ino a id sing!	acids	5							
25		(ii)	MOLI	ECULI	E TYI	PE: 1	prote	ein									
30		(xi)	SEQ	JENCI	E DES	SCRII	PTIO	N: S1	EQ II	ои с	:85:						
		Met 1	Tyr	Ile	His	Pro 5	Gln	Asn	Asn	Ser	Ile 10	Cys	Сув	Thr	Lys	Cys 15	His
35		Lys	Gly	Thr	Tyr 20	Leu	Tyr	Asn	Asp	Cys 25	Pro	Gly	Pro	Gly	Gln 30	Asp	Thr
		Asp	Суѕ	Arg 35	Glu	Cys	Glu	Ser	Gly 40	Ser	Phe	Thr	Ala	Ser 45	Glu	Asn	His
40		Leu	Arg 50	His	Cys	Leu	Ser	Cys 55	Ser	Lys	Суз	Arg	Lys 60	Glu	Met	Gly	Gln
45		Val 65	Glu	Ile	Ser	Ser	Cys 70	Thr	Val	Asp	Arg	Asp 75	Thr	Val	Cys	Gly	Cys 80
		Arg	Lys	Asn	Gln	Tyr 85	Arg	His	Tyr	Trp	Ser 90	Glu	Asn	Leu	Phe	Gln 95	Cys
50		Phe	Asn	Суз	Ser 100	Leu											
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5 5		(i)	(A (B (C	UENCI) LEI) TYI) STI) TOI	NGTH PE: 8 RANDI	: 91 amind EDNE:	amin o ac: SS: s	no ad id sing:	cids								
60		(ii)	MOL	ECULI	E. TYI	PE:]	prote	ein									

	(xi)	SEQU	JENCE	E DES	SCRIE	OITS	I: SE	EQ II	ONO:	:86:						
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	Gly	Pro	Gly	Gln 20	Asp	Thr	Asp	Cys	Arg 25	Glu	Суѕ	Glu	Ser	Gly 30	Ser	Phe
10	Thr	Ala	Ser 35	Glu	Asn	His	Leu	Arg 40	His	Cys	Leu	Ser	Cys 45	Ser	Lys	Cys
15	Arg	Lys 50	Glu	Met	Gly	Gln	Val 55	Glu	Ile	Ser	Ser	Cys 60	Thr	Val	Asp	Arg
	Asp 65	Thr	Val	Cys	Gly	Cys 70	Arg	Lys	Asn	Gln	Tyr 75	Arg	His	Tyr	Trp	Ser 80
20	Glu	Asn	Leu	Phe	Gln 85	Cys	Phe	Asn	Cys	Ser 90	Leu					
	(2) INFO	RMAT	ON I	FOR S	SEQ 3	D NO	0:87	:								
25	(i)	SEQU (A) (B) (C) (D)	LEI TYI STI	NGTH PE: 8 RANDI	ARACT 94 amino EDNES	amin ac: SS: s	no ad id sing:	cids								
30	(ii)	MOLI	ECULI	E TYI	PE: p	prote	ein									
35		SEQ		•												
	Met 1	Ser	Ile	Ser	Cys 5	Thr	Lys	Сув	His	Lys 10	Gly	Thr	Tyr	Leu	Tyr 15	Asr
40	Asp	Суѕ	Pro	Gly 20	Pro	Gly	Gln	Asp	Thr 25	Asp	Cys	Arg	Glu	Cys 30	Glu	Ser
45	Gly	Ser	Phe 35	Thr	Ala	Ser	Glu	Asn 40	His	Leu	Arg	His	Cys 45	Leu	Ser	Суз
15	Ser	Lys 50	Суз	Arg	Lys	Glu	Met 55	Gly	Gln	Val	Glu	Ile 60	Ser	Ser	Суѕ	Thr
50	Val 65	Asp	Arg	Asp	Thr	Val 70	Cys	Gly	Cys	Arg	Lys 75	Asn	Gln	Tyr	Arg	His 80
	Tyr	Trp	Ser	Glu	Asn 85	Leu	Phe	Gln	Cys	Phe	Asn	Cys	Ser	Leu		

WHAT IS CLAIMED IS:

- A chimeric polypeptide comprising an amino acid
 sequence of an osteoprotegerin dimerization domain fused to a heterologous amino acid sequence.
- The polypeptide of Claim 1 wherein the heterologous amino acid sequence and the
 osteoprotegerin dimerization domain are human.
- 3. The polypeptide of Claim 1 wherein the heterologous amino acid sequence and the osteoprotegerin dimerization domain are from different species.
 - ,4. The polypeptide of Claim 1 covalently associated with one or more chimeric polypeptides which result in a mulitmeric polypeptide complex.
 - 5. The polypeptide of Claim 4 wherein the complex is a dimer.
- 6. The polypeptide of Claim 1 wherein the heterologous amino acid sequence is a membrane-bound receptor lacking functional membrane associated amino acid sequences.
- 7. The polypeptide of Claim 6 wherein the receptor is selected from the group consisting of receptor tryrosine kinases, cytokine receptors, seven transmembrane domain receptors, and cell adhesion receptors.

- 8. The polypeptide of Claim 1 wherein the heterologous amino acid sequence is selected from members of the tumor necrosis factor-like receptor family consisting of TNFR-1, TNFR-2, TNFrp, NGFR, FasB, CD40, OX40, CD27, CD30, and 4-1BB.
- 9. The polypeptide of Claim 8 wherein the heterologous sequence comprises TNFR-1 lacking functional membrane-associated sequences.
 - 10. The polypeptide of Claim 9 wherein the heterologous sequence is a 30 kDa TNF inhibitor, a 40 kDa TNF inhibitor, or an analog thereof.

5

- 11. The polypeptide of Claim 1 wherein the carboxy terminus of the heterologous sequence is fused to the amino terminus of the OPG dimerization domain.
- 20 12. The polypeptide of Claim 1 wherein the amino terminus of the heterologous sequence is fused to the carboxy terminus of the OPG dimerization domain.
- 13. The polypeptide of Claim 1 wherein one or more 25 amino acids are inserted between the heterologous sequence and the OPG dimerization domain.
 - 14. A multimeric polypeptide comprising covalently associated monomers of OPG chimeric polypeptides.

- 15. The multimeric polypeptide of Claim 14 which is a dimer.
- 16. An isolated nucleic acid sequence encoding the 35 polypeptide of Claim 1.

- 17. An expression vector comprising the nucleic acid sequence of Claim 16.
- 18. A host cell transformed or transfected with the expression vector of Claim 17 in a manner allowing expression of the nucleic acid.
- 19. A pharmaceutical composition comprising the10 polypeptide of any of Claims 1 to 15.

1/8 FIGURE 1

Rat: Phe Phe		Ası	n <u>Cys</u>	g Gly	/ Ile	e Asp	Val	l Thi	c Lev	ı <u>Cys</u>	<u>G</u> lı	ı Glu	ı Ala	ì
Mouse:	Lys	<u>Cys</u>	Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	Phe	Phe
Human:	Lys	<u>Cys</u>	Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala	Phe	Phe
Rat:	Ara	Pho	7 T a	77-7	Dwo	шь	T	T1 _	T1_	D	_	_	_	_
Mouse:	Arg	Phe	Ala	Val Val	Pro	Thr	Lys	TIE	TIE	Pro	Asn	Trp	Leu	Ser
Human:	Ara	Phe	Ala	Val	Pro	Thr	Lys	Phe	The	Pro	Asn	Trp	Leu	Ser
	_						_1_				ASII	TTD	nea	Ser
n -4	**- 7		1	_	_			_						
Rat:	val	Leu	Val	Asp	Ser	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu
Mouse: Human:	Val	Leu	val	Asp	Ser	Leu	Pro	GLY	Thr	Lys	Val	Asn	Ala	Glu
numan:	val	ьеи	val	Asp	Asn	Leu	Pro	GIA	Thr	гàг	Val	Asn	Ala	Glu
Rat:	Ser	Val	Glu	Arg	Ile	Lys	Arg	Arg	His	Ser	Ser	Gln	Glu	Gln
Mouse:	Ser	Val	Glu	Arg	Ile	Lys	Ara	Ara	His	Ser	Ser	Gln	Glu	Gln
Human:	Ser	Val	Glu	Arg	Ile	Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln.
Rat:	Thr	Dho	Cln	Tou	T 011	T	T	Ф	T	77.5	6 7	_	_	_
Mouse:	Thr	Phe	Gln	Leu Leu	Leu	Lys	Leu	Trp	Lys	nis	Gin	Asn	Arg	Asp
Human:	Thr	Phe	Gln	Leu	Leu	Lvs	Leu	Trp	LVS	His	Gin	Asn	LVC	ASD
						-10		2	4,5	111.5	G111	USII	БУЗ	ASD
			•											
Rat:	Gin	Glu	Met	Val	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu	<u>Cys</u>
Mouse: Human:	Gin	GIU	Met	Val	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu	<u>Cys</u>
numan:	GIII	Asp	тте	Val	гдs	гЛS	тте	тте	Gin	Asp	Ile	Asp	Leu	<u>Cys</u>
Rat:	Glu	Ser	Ser	Val	Gln	Arg	His	Ile	Glv	His	Ala	Asn	Leu	Thr
Mouse:	Glu	Ser	Ser	Val	Gln	Arg	His	Leu	Gly	His	Ser	Asn	Leu	Thr
Human:	Glu	Asn	Ser	Val	Gln	Arg	His	Ile	Gly	His	Ala	Asn	Leu	Thr
										•				
Rat:	Thr	Glu	Gln	Leu	Ara	Tlo	T 011	Mot	C111	Com	T 011	Dwo	01:	T
Mouse:	Thr	Glu	Gln	Leu	Len	Ala	Leu	Met	Glu	Ser	Leu	Pro	Gly	Lys
Human:	Phe	Glu	Gln	Leu	Ara	Ser	Leu	Met	Glu	Ser	Leu	Pro	Glv	Lvs
													4 -1	2,0
D - F	•		~	_	_									
Rat: Mouse:	Lys	TIE	Ser	Pro	Asp	Glu	Ile	Glu	Arg	Thr	Arg	Lys	Thr	Cys
Human:	Lys	TTE	Ser	Ala	GIU	GIU	TIE	Glu	Arg	Thr	Arg	Lys	Thr	Cys
naman.	בעע	var	GTA	Ala	GIU	Asp	тте	GIU	гуѕ	THE	тте	гÀ2	Ата	<u>Cys</u>
Rat:	Lys	Pro	Ser	Glu	Gln	Leu	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg
Mouse:	Lys	Ser	Ser	Glu	Gln	Leu	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Ara
Human:	ьys	Pro	Ser	Asp	Gln	Ile	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg

FIGURE 1 (Con't)

Rat: Mouse: Human:	тте	ьуs	Asn	Gly	Asp	Gln	qzA	Thr	Leu	Lvs	Glv	Len	Met	Tyr Tyr His
Rat: Mouse: Human:	Ата	Leu Leu Leu	Lys	Hıs	Leu	Lys	Thr	Ser	His	Phe	Pro	Laze	Thr	17=1
Rat: Mouse: Human:	Thr	His His Gln	Ser	Leu	Arg	Lys	\mathtt{Thr}	Met	Arq	Phe	Leu	His	Ser	Phe
Rat: Mouse: Human:	Thr	Met Met Met	\mathtt{Tyr}	Arg	Leu	Tyr	Gln	Lys	Leu	Phe	Leu	Glu	Met	Tle
Rat: Mouse: Human:	GLY	Asn Asn Asn	Gln	Val	Gln	Ser	Val	Lys	Ile	Ser	Cvs	Leu		

FIGURE 2

30kDa TNF Inhibitor

5'-GA	TAG	TGT	GTG	TCC	CCA	AGG	AAA	ATA	TAT	CCA	.ccc	TCA	AAA	TAA	TTC	GAT	TTG	CTG	TAC	C-
D D	S	v	+	P	Q	G	+	Y	ī	-+- H	P	Q	+ N		 s		+		- <u>-</u> -	-
-AA	GTG	CCA	CAA	AGG	AAC	CTA	CTT	GTA	CAA	TGA	.CTG	TCC	AGG	ccc	GGG	GCA	GGA	TAC	GGA	C-
K	С	Н	K	G	T	Y	L	Y	N	D	С	P	G	P	G	Q	D	T	D	
-TG	CAG	GGA	GTG	TGA	GAG	CGG	CTC	CTT	CAC	CGC	TTC	AGA	AAA	CCA	CCT	CAG	ACA	.CTG	CCT	C-
C	R	E	C	E	S	G	s	F	T	-+ - А	s	E	N	H	L	R	+ H	C	L	- -
-AG	CTG	CTC	CAA	ATG	CCG	AAA	GGA	AAT	GGG	TCA	GGT	GGA	GAT	CTC	TTC	TTG	CAC	AGT	'GGA	C-
S	С	S	K	С	R	K	E	M	G	Q	v	E	I	s	s	C	+ T	V	D	-
-CG	GGA	CAC	CGT	GTG	TGG	CTG	CAG	GAA	GAA	CCA	GTA	.CCG	GCA	TTA	TTG	GAG	TGA	AAA	CCT	T-
Ŕ	D	т	V	С	G	C	R	K	N	Q	Y	R	H	Y	W	s	+	N	L	-
-TT	CCA	GTG	CTT	CAA	TTG	CAG	CCT	CTG	CCT	CAA	TGG	GAC	CGT	GCA	CCT	CTC	CTG	CCA	GGA	G-
F	Q	С	F	N	С	s	L	C	L	N	G	Т	v	Н	L	s	+	Q	E	-
-AA	ACA	GAA	CAC	CGT	GTG	CAC	CTG	CCA	TGC	AGG	TTT	CTT	TCT	AAG	AGA	AAA	CGA	.GTG	TGT	C-
K	Q	N	т	v	C	T	C	H	A	G	F	F	L L	R	E	N	+ E	C	v	-
-TC	CTG	TAG	TAA	CTG	TAA	.GAA	AAG	CCT	GGA	GTG	CAC	GAA	GTT	GTG	CCT	ACC	CCA	GAI	'TGA	G-
S	С	S	N	С	K	K	S	L	E	C	T	K	L L	C	L	P	+ Q	I	E	
-AA	T -3	•																		
Ŋ	*	-																		

FIGURE 3

40kDa TNF Inhibitor

L	P	Α	Q	V	Α	F	\mathbf{T}	P	Y	À	P	E	P.	G	S	$^{}$ T	C	R	L	_
AG	AGA	מיזמ	ርጥል	ጥር አ	ന്ന് മ	.GAC	አርር	יתורי א	CAT	cmc	СШС	C 2 C	~~ ~	ОШО	~~~					
+-			+				+			-+-			+				+	CCA	AAC	Į.
R	E	Y	Y	D	Q	T	A	Q	M	С	С	S	K	С	S	P	G	Q	Н	
GC	AAA	AGT	CTT	CTG	TAC	CAA	GAC	CTC	GGA	.CAC	CGT	GTG	TGA	CTC	CTG	TGA	.GGA	CAC	CA	C
т- А	ĸ	v	F	C	T	K	+ T	s	D	-+- T			+ D	 s		 E	+	 S	 Т	-
TA	CAC	CCA	GCT	CTG	GAA	.CTG	ഭവ	יייריר	ירכים	стс	டுரார	CAC	ריייכ	ጥርር	CTTC		C/II/C	·	-	_
+-			+				+			-+-			+				+	TAC		-
Y	T'	Q	L	M.	N	W	V	P	E	С	L	S	С	G	S	R	С	S	S	
GA	CCA	.GGT	GGA	AAC	TCA	AGC	CTG	CAC	TCG	GGA	ACA	GAA	.CCG	CAT	CTG	CAC	СТС	CAG	GC	(
D D	Q	v	E	T	Q	 А	+		R	-+- E			+ R	 I			+	R	 P	•
GG	CTG	GTA	СТС	CGC	ርርጥ	'GAG	ממי	CCA	CCA	GGG	כיייכ	ccc	CCM	C Π C					-	
			+				+			-+-			+				+			
~	T.7	7.5	~	_	-	_		_		_										
Ġ	W	Y	С	A	L	S	K	Q	E	G	С	R	L	С	A	P	L	R	K	
_		_				S CGT		~						_		_				
_		_						~						_		_				7
TG +- C	CCG R	CCC P	GGG + G	CTT F	CGG G	CGT V	GGC + A	CAG R	ACC P	AGG -+- G	AAC T	TGA E	AAC + T	ATC S	AGA D	.CGT V	GGT + V	GTC C	GCA K	7
TG +- C CC +-	CCG R CTG	CCC P	GGG + G CCC	CTT F GGG	CGG G G	CGT V GTT	GGC + A CTC +	CAG R	P CAC	AGG -+- G GAC	AAC T	TGA E ATC	AAC + T CAC	ATC S GGA	AGA D TAT	.CGT V	GGT + V	GTC C	GCA K	7
TG +- C	CCG R	CCC P	GGG + G	CTT F	CGG G	CGT V	GGC + A	CAG R	ACC P	AGG -+- G	AAC T	TGA E	AAC + T	ATC S	AGA D	.CGT V	GGT + V	GTC C	GCA K	2
TG +- C CC +- P	CCG R CTG	CCC P TGC	GGG + G CCC + P	CTT F GGG G	CGG G G GAC	CGT V GTT	GGC + A CTC +	CAG R CAA	P CAC	AGG -+- G GAC -+- T	AAC T TTC	TGA E ATC	AAC T T CAC	ATC S GGA	AGA D TAT	CGT V TTG	GGT + V CAG + R	GTC C GCC	GCA. K	7
TG +- C CC +- P	CCG R CTG	CCC P TGC	GGG + G CCC + P	CTT F GGG G	CGG G G GAC	CGT V GTT 	GGC + A CTC +	CAG R CAA	P CAC	AGG -+- G GAC -+- T	AAC T TTC	TGA E ATC	AAC T T CAC	ATC S GGA	AGA D TAT I	CGT V TTG C	GGT + V CAG + R	GTC C GCC	GCA. K	7
TG +- C CC +- P CA +- Q	CCG R CTG C GAT	P TGC A CTG	GGG + G CCC + P TAA TAA	CTT F GGG G CGT V	CGG GAC T GGT V	CGT V CGTT F CGGC	GGC + A CTC + S CAT + I	CAA R CAA N CCCC	P CAC T	GAC T GAC T GAA	AAC T TTC S TGC	TGA E ATC S AAG	AAC T CAC T CAG	ATC S GGA D GGA D	AGA D TAT I TGC	CGT V TTG C AGT	GGT V CAG + R CTG + C	GTC C GCC P	CCC.	7
TG +- C CC+- P CA +- Q	CCG R CTG C GAT	CCC P TGC A CTG C	GGG+ G CCC+ P TAA+ N CAC	CTT F GGG G CGT V	GAC T GGT GGT V	CGT V GTT F CGC A	GGC + A CTC + S CAT + I	CAA R CAA N CCCC	P CAC T TGG	GAACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	TTC S TGC A	TGA E ATC S AAG	AAC T CAC T CAG	ATC S GGA D GGA D	AGA D TAT I TGC	CGT V TTG C AGT	GGT V CAG + R CTG + C	GTC C GCC P	CCC.	7
TG +- C CC +- P CA +- Q	CCG R CTG C GAT	P TGC A CTG	GGG + G CCC + P TAA TAA	CTT F GGG G CGT V	CGG GAC T GGT V	CGT V CGTT F CGGC	GGC + A CTC + S CAT + I	CAA R CAA N CCCC	P CAC T	GAC T GAC T GAA	AAC T TTC S TGC	TGA E ATC S AAG	AAC T CAC T CAG	ATC S GGA D GGA D	AGA D TAT I TGC	CGT V TTG C AGT	GGT V CAG + R CTG + C	GTC C GCC P	CCC.	
TG+-CC+-PCAQAC+-T	CCG R CTG C GAT GTC	CCC P TGC A CTG C	GGG+ G CCC+ P TAA+ N CAC	CTT F GGG G CGT V CCG	GAC T GGT GGT V GAG	CGT V GTT F CGC A	GGC + S CAT + I GGC +	CAA R CAA N CCCC P	ACC P CAC T T G	GAA GAA GAA H GGAA H GGGC	AAC TTC S TGC A AGT	TGA E ATC S AAG ACA H	AAC + T CAC + T CAG + R	ATC S GGA D GGA D ACC	AGA TAT I TGC A	CGT V TTG C C AGT V	CAG + R CTG + C	GTC C GGCC P GCAC T	GCA K CCCC. H CGTT S	7
TG+-CC+-PCATCG+-	CCG R CTG C GAT GTC S ATC	CCC P TGC A CTG C CCC	GGG+ G CCC+ P TAA+ N CAC+ ACA	CTT F GGG G CGT V CCG R CAC	GAC T GAG GAG GAG	CGT V GGTT F GGC A TAT	GGC + S CTC + S CAT + I GGC + A	CAA R CAA N CCCC P	P CAC T TGG	GAC GAC T GAA GAA -+- N GGC -+- A	TTC S TGC A AGT V	TGA E ATC S AAG AAG H CACA	AAC+ T CAC+ R CTT L	ATC S GGA D GGA D ACC P	AGA TAT I TGC A CCA Q AAG	CGT V TTG C AGT V GCC	CAG + R CTG + C	GTC C GGCC P GCAC T	GCA. KCCC. H CGT S CCA T	7
TG+-CC+PCA-TCG+R	CCG R CTG C GAT GTC S ATC	CCC P TGC A CTG CCC	GGG GCC+ P TAA+ N CAC+ T ACA	CTT F GGG G CGT V CCG R CAC	GAC GAG GAG GAG	CGT V GTT F GGC A TAT	GGC + A CTC + S CAT + I GGC + A AAC	CAA R CAA N CCCC	P CAC T TGG AGG	GAAC GGAC ACC ACC P	AAC TTC S TGC A AGT V CAG	TGA E ATC S AAG ACA H	AAC+ T CAC+ R CTT+ L	ATC S GGA D GGA D TCC	AGA D TAT I TGC A CCA Q AAG	CGT V TTG C AGT V .GCC	CAG + R CTG + C	GTC C GGCC P GCAC T	GCA. KCCC. H CGT S CCA T	7

FIGURE 4

				•	
	1				50
TNFbp/OPG	MGLSTVPDLL	LPLVLLELLV	GTYPSGVTGL	VPHI CDPEKP	DSVCPQGKYI
TNFbp 4.0	MGLSTVPDLL	LPLVLLELLV	GIYPSGVIGL	VPHLGDREKE	DSVCPQGKYI
TNFbp/196	MGLSTVPDLL	LPLVLLELLV	GTYPSGVTGL	VPHI.CDPEKP	
TNFbp/217	MGLSTVPDLL	LPLVLLELLV	GIYPSGVIGL	VILLEGORERE	DSVCPQGKYI
TNFbp/248		LPLVLLELLV			DSVCPQGKYI
TNFbp/304	MCLSTVPDLL	LPLVLLELLV		VPHLGDREKR	DSVCPQGKYI
1111 20 7 3 0 4	TIONOT AT DUD	TE TATTETTA	GIIFSGAIGE	VPHLGDREKR	DSVCPQGKYI
	51				
TNFbp/OPG		WOULD OF THE	DGDGDGGD	~~~~	100
TNFbp 4.0	HEOMNGTCCT	KCHKGTYLYN		CRECESGSFT	ASENHLRHCL
		KCHKGTYLYN	~	CRECESGSFT	ASENHLRHCL
TNFbp/196	HPQNNSICCT	KCHKGTYLYN	DCPGPGQDTD	CRECESGSFT	ASENHLRHCL
TNFbp/217	HPQNNSICCT	KCHKGTYLYN	DCPGPGQDTD	CRECESGSFT	ASENHLRHCL
TNFbp/248		KCHKGTYLYN	DCPGPGQDTD	CRECESGSFT	ASENHLRHCL
TNFbp/304	HPQNNSICCT	KCHKGTYLYN	DCPGPGQDTD	CRECESGSFT	ASENHLRHCL
	101				150
TNFbp/OPG	SCSKCRKEMG	QVEISSCTVD	RDTVCGCRKN	QYRHYWSENL	FQCFNCSLCL
TNFbp 4.0	SCSKCRKEMG	QVEISSCTVD	RDTVCGCRKN	QYRHYWSENL	FQCFNCSLCL
TNFbp/196	SCSKCRKEMG	QVEISSCTVD	RDTVCGCRKN	QYRHYWSENL	FQCFNCSLCL
TNFbp/217	SCSKCRKEMG	QVEISSCTVD	RDTVCGCRKN	QYRHYWSENL	FQCFNCSLCL
TNFbp/248	SCSKCRKEMG	QVEISSCTVD	RDTVCGCRKN	QYRHYWSENL	FOCFNCSLCL
TNFbp/304	SCSKCRKEMG	QVEISSCTVD	RDTVCGCRKN	QYRHYWSENL	FQCFNCSLCL
-		2	1.51 / 0001.141	ZIIGIIWODIND	TOCTMODECT
	151				200
TNFbp/OPG	NGTVHLSCQE	комплотона	GFFLRENECV	SCSNCKKSLE	CTKLCLPQIE
TNFbp 4.0	NGTVHLSCQE		GFFLRENECV	SCSNCKKSLE	CTKLCLPQIE
TNFbp/196	NGTVHLSCQE		GFFLRENECV	SCSNCKKSLE	
TNFbp/217	NGTVHLSCQE		GFFLRENECV		CTKLCLPQIE
TNFbp/248	NGTVHLSCQE		GFFLRENECV	SCSNCKKSLE	CTKLCLPQIE
TNFbp/304	NGTVHLSCQE			SCSNCKKSLE	CTKLCLPQIE
IMP.DD/304	MGIAUDSCOF	KONTVCTCHA	GFFLRENECV	SCSNCKKSLE	CTKLCLPQIE
	201				
MATERN /ODG		M 01/0075			250
TNFbp/OPG	NVKGTEDSGT	TGKCGIDVTL	CEEAFFRFAV	PTKFTPNWLS	VLVDNLPG:
TNFbp 4.0	NVKGTEDSGT	T	• • • • • • • • • •	• • • • • • • • • •	• • • • • • • • •
TNFbp/196	NVKGTEDSGT	TGIDVTL	CEEAFFRFAV	PTKFTPNWLS	VLVDNLPG7
TNFbp/217	NVKGTEDSGT	TG	• • • • • • • • •	PNWLS	VLVDNLPG T
TNFbp/248	NVKGTEDSGT	TG	• • • • • • • • •		
TNFbp/304	NVKGTEDSGT	TG			
		196 (OP	G)	217 (0:	PG)
					•
	251				300
TNFbp/OPG	VNAESVERIK	RQHSSQEQTF	QLLKLWKHON	KDQDIVKKII	ODIDLCENS.
TNFbp 4.0					
TNFbp/196	VNAESVERIK	RQHSSOEOTF	OLLKLWKHON	KDODIVKKII	ODIDLCENS:
	VNAESVERIK	ROHSSOEOTF	OLLKLWKHON	KDODIVKKIT	ODIDLOFNE
TNFbp/248	• • • • • • • •	EQTF	OLTKI MKHON	KDODIVKKIT	ODIDI GENG.
TNFbp/304.		* * * * * * * * * * *	5 mm m m m 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1		ろって ヘコパ カオイベ
• • • • •	,	248 (OPG)	• • • • • • • • • •	• • • • • • • •
	•	230(or G)		

TNFbp/OPG TNFbp 4.0 TNFbp/196 TNFbp/217 TNFbp/248 TNFbp/304	301 QRHIGHANLT QRHIGHANLT QRHIGHANLT QRHIGHANLT	FEQUESTMES FEQUESTMES FEQUESTMES FEQUESTMES	LPGKKVGAED LPGKKVGAED LPGKKVGAED LPGKKVGAED PGKKVGAED 304 (OPG)	IEKTIKACKP IEKTIKACKP IEKTIKACKP IEKTIKACKP IEKTIKACKP	350 SDQILKLLSI SDQILKLLSI SDQILKLLSL SDQILKLLSL SDQILKLLSL
TNFbp/OPG TNFbp 4.0 TNFbp/196 TNFbp/217 TNFbp/248 TNFbp/304	WRIKNGDQDT WRIKNGDQDT WRIKNGDQDT WRIKNGDQDT WRIKNGDQDT WRIKNGDQDT	LKGLMHALKH LKGLMHALKH LKGLMHALKH LKGLMHALKH LKGLMHALKH	SKTYHFPKTV SKTYHFPKTV SKTYHFPKTV SKTYHFPKTV SKTYHFPKTV	TOSLKKTIRF TOSLKKTIRF TOSLKKTIRF TOSLKKTIRF TOSLKKTIRF	400 LHSFTMYKLY LHSFTMYKLY LHSFTMYKLY LHSFTMYKLY LHSFTMYKLY
TNFbp/OPG TNFbp 4.0 TNFbp/196 TNFbp/217 TNFbp/248 TNFbp/304	401 QKLFLEMIGN QKLFLEMIGN QKLFLEMIGN QKLFLEMIGN QKLFLEMIGN	420 QVQSVKISCL QVQSVKISCL QVQSVKISCL QVQSVKISCL QVQSVKISCL	01 (OPG)		



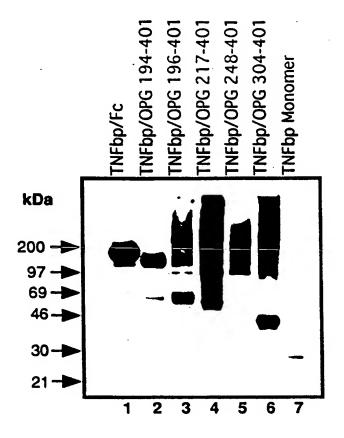
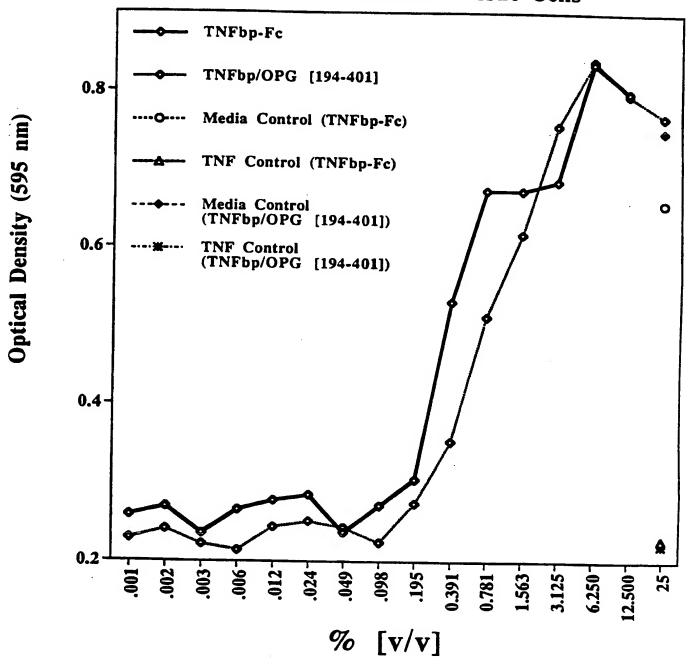


FIGURE 6

Inhibition of TNF Cytotoxicity of L929 Murine Connective Tissue Cells



INTERNATIONAL SEARCH REPORT

Internati Application No PCT/US 98/08631

		1 1017 00 307	7 00031	
IPC 6	FICATION OF SUBJECT MATTER C12N15/12 C12N15/62 C07K14/7	705 A61K38/17		
According to	o International Patent Classification (IPC) or to both national classifica	ation and IPC		
B. FIELDS	SEARCHED			
IPC 6	ocumentation searched (classification system followed by classification CO7K C12N			
	tion searched other than minimumdocumentation to the extent that s at a base consulted during the international search (name of data ba		·	
Listionicu	and base consumed during the litternational search (name of data ba	se and, where practical, search terms used)		
C. DOCUMI	ENTS CONSIDERED TO BE RELEVANT			
Category °	Citation of document, with indication, where appropriate, of the rele	evant passages	Relevant to claim No.	
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Y	& WO 96 26217 A (SNOW BRAND MILK 29 August 1996	PRODUCTS)	1-19	
Υ	EP 0 526 905 A (YEDA RES & DEV) 10 February 1993 See, in particular, example 3d.		1–19	
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		-/	-	
	her documents are tisted in the continuation of box C.	X Patent family members are listed in	n annex.	
	tegories of cited documents :	"T" later document published after the inter or priority date and not in conflict with	rnational filing date	
considered to be of particular relevance "E" earlier document but published on or after the international filing date "X" document of particular relevance; the of			eory underlying the	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "Cannot be considered novel or cannot involve an inventive step when the document of particular relevance; the constant be considered novel or cannot be considered nov			cument is taken alone	
"O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but "Carntot be considered to involve an invol			ore other such docu- us to a person skilled	
	actual completion of theinternational search	"&" document member of the same patent		
	O September 1998	Date of mailing of the international search report 25/09/1998		
Name and n	nailing address of the ISA European Patent Office, P.B. 5818 Patentiaan 2	Authorized officer		
	NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+3 <u>1</u> -70) 340-3016	Lonnoy, O		

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Internat: Application No PCT/US 98/08631

		T/US 98	3/08631
	ation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages		Relevant to claim No.
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